STUDIES ON QUINONES

Part I. The Structure of Fuerstiaquinone.

Part II. The Structure of Carminic acid.

By

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A C K N O W L E D G E M E N T

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C O N T E N T S

PART I.

The Structure of Fuerstiaquinone.

INTRODUCTION

In 1921 T.C.E. Fries discovered a plant[1] in East Africa which could not be classified into any of the known genera of the Labiatae family to which it belonged. The plant was considered by its discoverer to be of a new genus which he named Fuerstia (in honour of Prof. C.M. Fürst of Lunds University) and the species was accordingly called Fuerstia africana.

Fuerstia africana T.C.E. Fries is widely distributed in East Africa. It is found in abundance in Uganda, Kenya, and on Mt. Kilimanjaro in Tanganyika. The natives believe that this plant contains one or more principles which stimulate milk secretion and the Chagga tribe of Kilimanjaro actually use the plant for this purpose in case of humans as well as animals. The natives also use the leaves of this plant for colouring butter.

This property inspired P. Karrer and C.H. Eugster[2] to undertake a chemical investigation on this plant about 1951. On microscopic examination they found red droplets of a pigment on the lower surface of the leaves and on the calyx of the flowers. The dried plant material gave, with all common organic solvents, a red extract which contained a large proportion of other substances, in addition to the colouring matter. After preliminary purification of the extract and the application of chromatography they were able to isolate a deep red crystalline compound (m.p. 100-125°; yield 0.25-0.3%).

Karrer and Eugster observed that the red colour disappeared on treatment with a mild reducing agent, like dithionite, and, on aerial oxidation, the original colouration was restored. This fact led them to believe that the substance they were dealing with was a quinone and accordingly they named the compound as Fuerstiaquinone.[3]

According to these workers, Fuerstiaquinone quickly undergoes molecular rearrangement in presence of acids or alka-:lies to produce colourless solutions. It reduces ammoniacal $AgNO_3$ very readily, decolourises Tillman's reagent, and also gives different colour reactions with reagents like $FeCl_3$, $TiCl_3$ and KCN. The nature of this compound is best described in the words (translated) of Karrer and Eugster: ".....From these and other observations, it is clear that the new pigment possesses great instability and chemical reactivity which make its investigation more difficult. This compound seems to be of a type which essentially differs from all the hitherto known natural colouring matters.

Fuerstiaquinone is a typical reddish-yellow dye for fats and lipoids. Its application in colouring butter by the natives of East Africa is, therefore, based on true observation".

From the results of combustion analysis and molecular weight determination, Karrer and Eugster suggested the empirical molecular formula, $C_{20}H_{26}O_3$, for Fuerstiaquinone. ($C_{20}H_{26}O_3$ requires C, 76.40: H, 8.34. Found: C, 76.49; H, 8.48. Mol.

wt. of $C_{20}H_{26}O_3$ is 314. Found mol. wt. : 309 [camphor], 295 [benzene]). They also determined the ultra-violet absorption spectra of Fuerstiaquinone and its acid and alkali rearrangement products, but did not make any comment on them. From the fact that Fuerstiaquinone gave a deep red colour with $TiCl_3$ (enediol test) and formed a green crystalline copper complex, they suggested the presence of an ortho-hydroxyquinone group in its molecule.

Two years after the publication of Karrer's paper on Fuerstiaquinone, the Colonial Products Research Council, London became interested in this compound and obtained a large supply of the dried plants which they provided for this investigation. The present studies were commenced in 1955 in an attempt to elucidate the structure of Fuerstiaquinone.

ISOLATION

Karrer's method of isolation gave neither consistent nor satisfactory results when applied to the material at our disposal. Modifications described below led to the successful isolation of crystalline Fuerstiaquinone on each occasion on which they were employed. Karrer isolated Fuerstiaquinone in the following way:

Powdered dry leaves (350 g.) of Fuerstia africana were extracted twice with cold benzene (4 litres) and the solvent was removed from the filtered extract under reduced pressure. The viscous dark red residue thus obtained was extracted thrice with boiling ligroin (b.p. 30-60°) under reflux for 30 minutes. The resulting solution was decanted from the insoluble material and cooled in the refrigerator yielding an insoluble resin which was discarded. The supernatant solution was concentrated (to 400 ml.) and partitioned with 95% methanol (4 x 100 ml.). The colouring matter passed into the methanol layer which was separated; removal of all solvent from the methanol extract under reduced pressure gave a residue which was a very thick dark red oil. This was dissolved in ligroin and chromatographed on a column of gypsum ($CaSO_4$, $\frac{1}{2}H_2O$). On eluting with ligroin, the pigment moved slowly as a diffuse red zone. The eluate was collected in fractions (ca. 100 ml.). The residues from these fractions which showed a log $E_{1\,cm.}^{1\%}$ greater than 2.2 at 440 mμ were combined and chromatographed again in the similar way on a magnesium sulphate (anhydrous) column. The fractions

which had a log $E^{1\%}_{1\ cm.}$ 2.35 at 440 mμ were again collected and concentrated to 10 ml. The concentrated ligroin solution was then cooled to - 70° while the walls of the container were rubbed with a glass rod. By doing this, the solution set to a gelatinous mass. The rubbing with the glass rod was con-:tinued until the solution attained room temperature again. After these operations had been repeated several times, Fuerstia-:quinone began to crystallise in the form of a fine scarlet-red, microcrystalline powder. The crystallisation was completed by immersing the solution in ice-water for some time. Other fractions from the chromatographic column, after concentration, were inoculated with the micro-crystals of Fuerstiaquinone and cooled; more crystals were thus obtained. Total yield was 640 mg. (0.18%).

Later Karrer found that instead of chromatographing through gypsum followed by magnesium sulphate, satisfactory results were obtained more directly by chromatography of the impure ligroin solution using a column of basic magnesium carbonate (Magnesium subcarbonicum Ph. H.V.) with ligroin as eluting solvent. The red middle-fraction of the eluate, on concentration and inoculation, yielded crystals of Fuerstiaquinone. According to him, if pure Fuerstiaquinone solution is chromatographed on basic magnesium carbonate, the pigment firmly lakes with the adsorbent in the form of a broad blue band which can be eluted unchanged with ligroin containing acetone; but in the case of

impure Fuerstiaquinone solution, the impurities are held more strongly at the top of the column and so, if a column of the right dimension is employed, the Fuerstiaquinone band can be washed out directly.

It is interesting to note that Karrer on one occasion was able to isolate Fuerstiaquinone from the plant without using chromatography, but he could not repeat this experiment with another sample which was collected in a different season. The process that he adopted for this was:

The plant material (2.87 kg.) was extracted once with cold and twice with hot benzene. The extract was concentrated to 3 litres under reduced pressure and allowed to stand for some time. A brown powdery mass separated that was removed by fil-:tration. The filtrate was concentrated (to 500 ml.) under vacuum and was mixed with ligroin (1 litre, b.p. 30-60°) with stirring. The precipitated resins were removed and the red solution was partitioned with 12 quantities of 95% methanol tot-:alling 1.5 litres. The combined methanolic solution was washed once with 200 ml. ligroin and then the solvent was removed under vacuum. The residue was completely dried at an oil pump pressure and the coloured resin thus obtained was boiled four times with 500 ml. ligroin (30 mins. each time). The combined ligroin extract was concentrated to 200 ml. and seeded with crystals of Fuerstiaquinone. On doing this, crude viscid crystals of Fuerstiaquinone were obtained. These were finally washed with cold

ligroin and collected.

Karrer recommended the solvents, cyclohexane, methyl cyclohexane, and iso-octane for recrystallisation of Fuerstiaquinone. He noted that, if cyclohexane is used, the resulting crystals contain bound cyclohexane and on analysis the empirical formula corresponds with $C_{20}H_{26}O_3$, $\frac{1}{3}$ C_6H_{12}.

When we attempted isolation of the pigment, we started with 350 g. of the plant powder, extracted with 4 litres of cold benzene and then proceeded exactly in the same manner described by Karrer. After preliminary purification of the extract, we chromatographed the impure ligroin* solution on a column of anhydrous magnesium sulphate using ligroin for elution. The separation was not found satisfactory. The eluate was collected in fractions of ca. 50 ml. Those fractions which looked intense red were combined (150 ml.) and were again chromatographed on magnesium sulphate in a similar way. The middle fraction, which was an intense red colour was concentrated to ca.10 ml. This was then cooled in a mixture of solid CO_2 and acetone and rubbed with a glass rod. Rubbing was continued until the solution attained room temperature. On repeating this process of cooling and rubbing several times, no crystals of Fuerstiaquinone appeared, only a dark red gum which, on keeping in air, solidified to a brittle resin. However, the solution was freed from the gum by filtration and then concentrated (ca.5 ml.). Cooling in dry ice/acetone and scratching with a glass rod gave more

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* The boiling range should always be understood as 40-60°, unless otherwise stated.

resin. This was removed and the same operation repeated several times until the solution had lost most of its colour. No crystalline material was obtained.

We started with fresh extracts of the powdered plant and repeated the whole process many times, but in every case we invariably obtained the dark red gum.

Next we turned our attention to basic magnesium carbonate. We found two varieties of basic magnesium carbonate in the British Pharmacopoeia i.e., Magnesii Carbonas Levis (light magnesium carbonate) and Magnesii Carbonas Ponderosus (heavy magnesium carbonate). These two varieties were available to us. On chromatographing the crude ligroin extract on a column of heavy magnesium carbonate and eluting with ligroin, the separation of the pigment from other impurities was found to be better; but in this case also, the eluate did not yield crystals by the cooling and scratching method. Repeated attempts failed and each time gum-like material was obtained.

We then activated heavy magnesium carbonate by heating at 120° for eight hours. Chromatography with this activated sample gave the same result as before. In this case the bands moved very slowly on eluting with ligroin. Mixtures of benzene and ligroin were also found unsatisfactory.

In the case of light magnesium carbonate, we experienced

that during chromatography the column invariably cracked distorting all the bands; but none the less the separation was very good. The blue band of Fuerstiaquinone moved much faster than all other bands due to impurities. The cracking of the column was due to the extreme lightness of the adsorbent. Different sizes of the columns as well as compact packing under pressure were tried but they were all of little use. A column made up from a mixture of heavy and light magnesium car-:bonate (1:1) also cracked during chromatography. When the proportion of heavy magnesium carbonate was increased then a good separation did not result. However, in one case we were successful in eluting the undistorted Fuerstiaquinone band from the light magnesium carbonate column. A thin column (15x2.1 cm.) was very compactly packed under suction (water pump). The impure ligroin solution was carefully put on the column and sucked by means of a water pump. Elution under suction was continued with ligroin. Though the column slightly cracked towards the end, still about 50 ml. of the eluate could be collected. This was a clean, intensely red solution. It was concentrated to a small volume (ca. 2 ml.) and allowed to stand. Within half an hour we found, to our surprise, Fuerstiaquinone spontaneously crystallising out from the solution.

Once the crystals of Fuerstiaquinone were obtained we found that the eluates from the heavy magnesium carbonate column

also yielded crystals spontaneously. As a matter of fact we never directly seeded a solution to obtain crystals of Fuerstia-quinone. The laboratory was in effect seeded.

In order to minimise time involved in the isolation we, later on, tried to purify the methanol solution by partition with benzene or ligroin instead of chromatography; but these were not successful. However, by methods of trial and error we eventually evolved the method described below, which in our hands is most satisfactory.

The dried leaves and twigs of Fuerstia africana were powdered in a hammer mill using a $\frac{1}{16}$" mesh screen. The powder was introduced into a long column (133 x 6.8 cm.) in portions sufficient to fill only 15-20 cm. of its length. After each addition the powder was firmly pressed with a ram-rod. In this way a tightly packed column (125 x 6.8 cm.) containing 1.092 kg. of the powder was made. The tap of the column was then opened and benzene was allowed to percolate slowly through the column. When 2 litres of the extract was collected, addition of benzene was stopped. The benzene adsorbed in the powder was allowed to drain out. Total volume of the extract was 3.5-4 litres. (If the plant material is not powdered as finely as mentioned above, then a compact column could not be prepared and complete extraction of the colouring matter could not be achieved even with 8 litres of benzene).

The benzene extract was then distilled under reduced

pressure until a small amount of benzene (5-10 ml.) remained in the flask. The dark red residue was extracted by shaking with cold ligroin (1 x 500 ml., 3 x 250 ml.). The insoluble material left was a dark brown solid mixed with some fine white amorphous powder which was not investigated. The combined ligroin extract was cooled in the refrigerator overnight and some brownish yellow powdery resin was deposited. This was removed by filtration and the filtrate was partitioned twice with 95% aqueous methanol (~~1 x 200,~~ 1 x 100 ml.). The combined methanolic solution was washed with 150 ml. portions of ligroin until the ligroin layer was slightly red (2-3 times). The methanolic solution was then evaporated to complete dryness under reduced pressure. A very viscous dark red oil was obtained which was not easily soluble in ligroin. The colouring matter was extracted from the resinous oil by shaking thrice with 500 ml. portions of ligroin. The ligroin extracts were combined and concentrated to a small volume (70-80 ml.) under reduced pressure. On standing for some time, some resin separated which was removed. The clear solution was then chromatographed on a column (15 x 5.5 cm.) of heavy magnesium carbonate. The blue band of Fuerstiaquinone, which moved fastest, was eluted with ligroin. The elution was stopped when the red colour of the eluate became very weak. In all ca. 200 ml. of the eluate was collected. This was then concentrated to a very small volume (ca. 1.5-2 ml.) and the

resulting intensely red oil deposited crystals of Fuerstiaquinone on standing. This was left overnight. Next day the mother-liquor was decanted and the crystals were washed with ligroin and collected. Total yield was 625 mg. (.057%).

The low yield, compared to that obtained by Karrer, is not due to incomplete extraction of the colouring matter from the plant, because we found that an 8 litre extract of the same amount of powder did not give a better yield of Fuerstiaquinone. The causes of low yield might be the following:

(1) In our extractions the powder contained twigs as well. On microscopic examination, we found very small proportion of the pigment to be present on the twigs compared to that on the leaves.

(2) The collection of the plant in a different season and at a different place.

(3) Extraction after a longer time from the time of collection.

On recrystallising Fuerstiaquinone from decalin, we found that the crystals (m.p. 105-6°) cannot be easily freed from the solvent. We found iso-octane (2:2:4-trimethylpentane) to be the best solvent for recrystallisation in agreement with Karrer.

It was observed that Fuerstiaquinone was only isolated

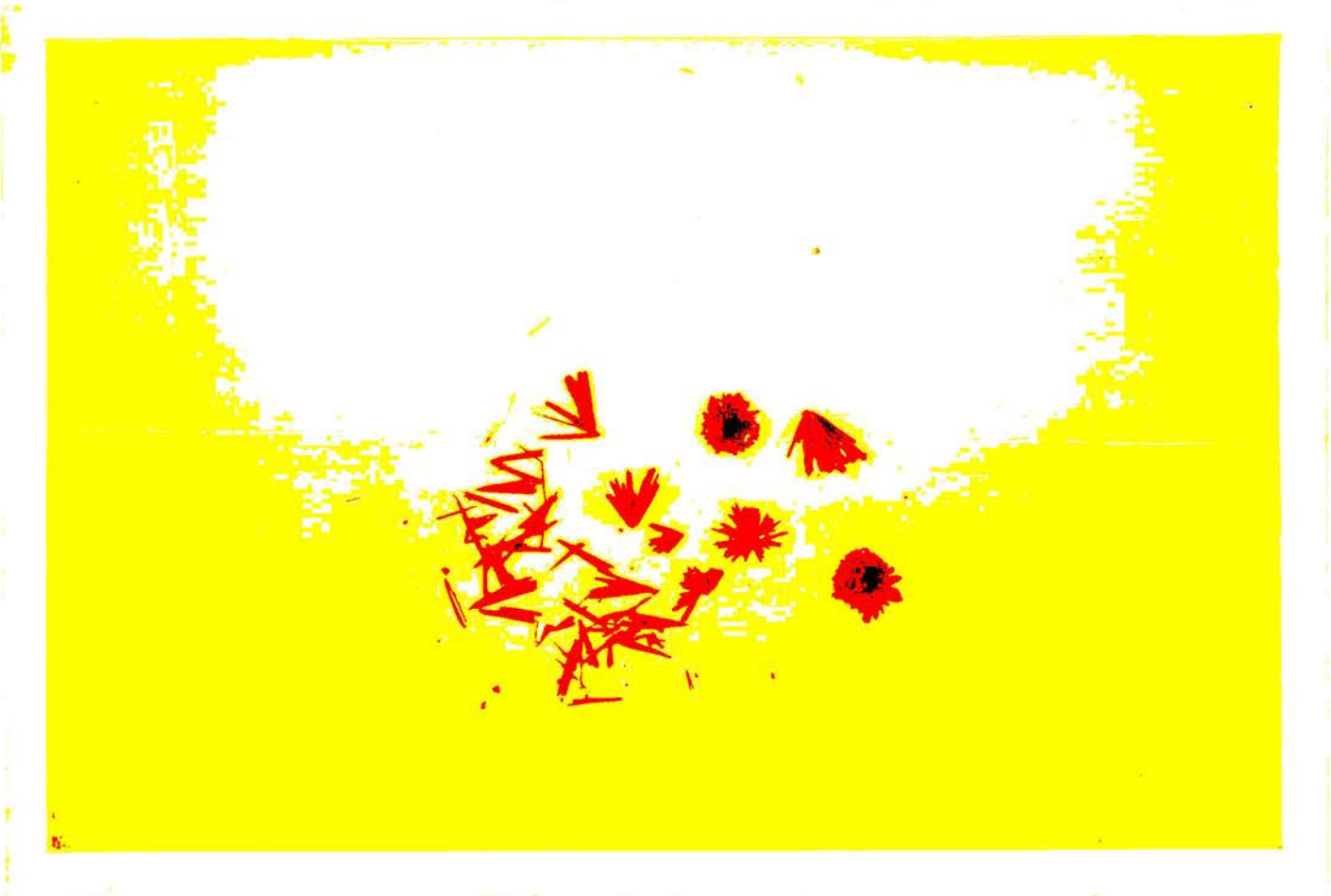
from the first four fractions (each 50 ml.) of the red eluate. The fifth and last red fraction, although not apparently different from those first eluted, gave dark almost black prisms on con-: centration. We considered this substance to be a possible dimer or a low polymer of Fuerstiaquinone.

According to our experience, Fuerstiaquinone is only stable after it has been recrystallised from iso-octane. This removes the last traces of a resinous substance which exerts a promotary effect on the polymerisation of the quinone. If the crystals isolated from ligroin are not thus treated they spon-: taneously blacken and one sample which had stood only for a few weeks gave less than 2% of the quinone after chromatographic separation from the polymer.

In the plant Fuerstiaquinone is reasonably stable and does not undergo such changes so quickly. We found that 1.092 kg. of the plant powder yielded 625 mg. of the pigment at one time and the same quantity of powder, after one year, gave 392 mg. of Fuerstiaquinone. It seems that some sort of protecting agent is present in the plant or Fuerstiaquinone is loosely bound to some material which inhibits polymerisation. A pure specimen of Fuerstiaquinone, prepared by recrystallising from iso-octane, is indefinitely stable in light and in air; one such sample remains completely unchanged after two years.

On slowly evaporating a solution of pure Fuerstiaquinone in iso-octane, we obtained some single crystals. These were

beautiful, deep red, long prismatic needles with light green reflex.



Single Crystals of Fuerstiaquinone

Bacteriological tests (by Dr. J.C. Gould, Bacteriology Department, University of Edinburgh) have shown that Fuerstiaquinone possesses some antibacterial activity though this is not of a high order. It has been found to inhibit Gram-positive organisms more readily than Gram-negative organisms.

Staphylococcus aureus was inhibited at a concentration of

about 1:20,000; a strain of Streptococcus pyogenes by 1:30,000 and Pneumococcus by 1:25,000. Escherichia coli was inhibited by 1:1500, but Pseudomonas and Proteus species were not.

These figures refer to reasonably large inocula but of course the inhibitory concentration will vary considerably with the size of the inoculum.

STRUCTURAL INVESTIGATION

Analyses of freshly prepared Fuerstiaquinone (thrice recrystallised from iso-octane, m.p. 108-109°) gave results in accordance with Karrer's empirical formula, $C_{20}H_{26}O_3$. (Found: C, 76.34, 76.07; H, 7.95, 8.07. Calc. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34). On evaporating a solution of Fuerstiaquinone in iso-octane very slowly in air for about four weeks some single crystals were deposited. These showed complete extinction under the polarising microscope. An X-ray molecular weight determination (by Dr. C.A. Beevers) has given a value of 318±6 which fully confirms Karrer's formulation.

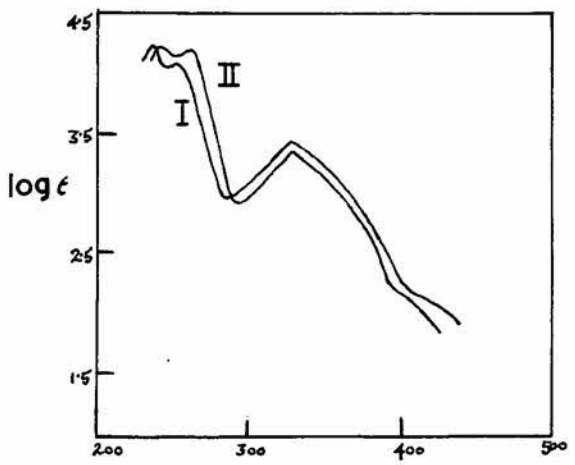
Fuerstiaquinone was found to contain no methoxyl group and at least one C-methyl group. It did not dissolve in aqueous sodium hydroxide (crystals turned black) or in aqueous acids, and also did not show any colour when in solution in concentrated sulphuric acid. In methanolic solution Fuerstiaquinone quickly underwent molecular rearrangement with acids and alkalies giving colourless solutions. With sodium hydrogen sulphite it only produced a colourless solution. Fuerstiaquinone gave a positive Dimroth test[4] for o-hydroxyquinones and also showed dark green and red colourations with $FeCl_3$ and $TiCl_3$ respectively.

Upon ordinary or catalytic hydrogenation, Fuerstiaquinone gave completely colourless solutions which gradually oxidised to red solutions on exposure to air. In the case of catalytic

hydrogenation, it was observed that absorption of one molecule of hydrogen made the substance colourless. Karrer has recorded data on catalytic hydrogenation using different catalysts and has concluded from them that in all cases one molecule of hydrogen was absorbed to render the quinone colourless.[3]

The ultra-violet and visible absorption spectrum of Fuerstiaquinone (Fig.I) showed an inflexion at 260 mμ (log ε 3.4) and a broad band with a maximum at 435 mμ (log ε 4.1), in agreement with Karrer.

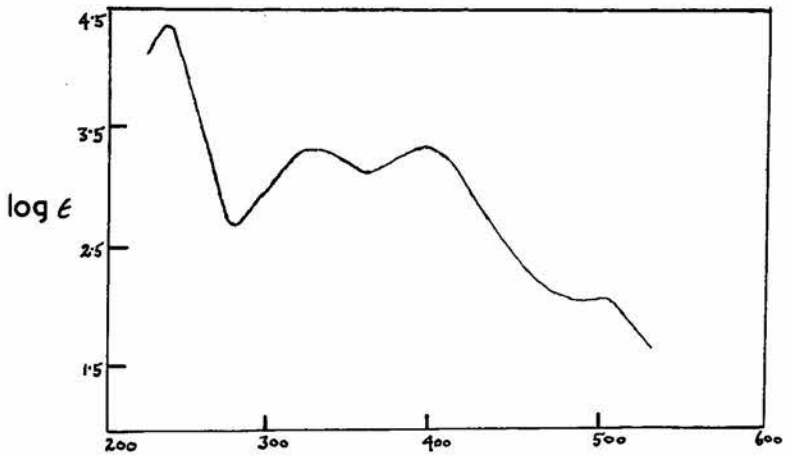
It is known that ortho- and para-quinones give characteristic ultra-violet absorption spectra. Macbeth et al.[5] have determined the spectra (shown below) of 1:4-naphthaquinone (I) and 2-methyl-1:4-naphthaquinone (II) typical of the 1:4-structure.



I. $\lambda_{max.}$ 246, 256 and 334 m (log ε 4.28, 4.13 and 3.44 respectively).

II. $\lambda_{max.}$ 248, 259 and 333 m (log ε 4.27, 4.23 and 3.37 respectively).

The spectrum of β-naphthaquinone (III), typical of the 1:2-structure, has been determined by Goldschmidt and Graef.[6]

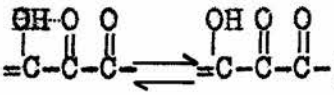


$\lambda_{max.}$ 250, 340, 400 and 500 mμ
(log ε 4.35, 3.40, 3.40 and 2.00 respectively).

From the above illustrations, the difference between the absorption due to 1:2-structure and that due to the 1:4-structure is clear. The band (λ ca. 335 mμ) of the 1:4-naphthaquinone, attributed to the carbonyl group in the system C=C-C=O, is still present in the 1:2-compound but an additional band at longer wavelength (λ ca.400 mμ) is also present. It seems, therefore, probable that the latter could be regarded as a criterion of 1:2-naphthaquinone structure (indeed of the α-dicarbonyl structure). Macbeth, Price and Winzor[5] state that the definite maximum in the region 400 mμ, and the inflexion in the region 490-500 mμ, are without parallel in the absorption characteristic of the 1:4-naphthaquinone type, and may reasonably be applied as a criterion to distinguish between 1:2- and 1:4- structure.[7]

At first sight a similarity exists in the shape of the ultra-violet and visible absorption curves of Fuerstiaquinone and those of substituted o-benzo and o-naphthaquinones.

But the intensity of the visible maximum at 435 mμ, in case of Fuerstiaquinone, is greater than those of the o-quinones[8] by a factor of nearly ten, a fact which seems to preclude this structural analogy. Another feature of the spectrum of Fuerstiaquinone which cannot be correlated with those of ortho-quinones is the absence of any strong absorption at shorter wavelengths apart from the inflexion at 260 mμ (cf. the spectra of Dunnione,[9] β-lapachone,[9] β-naphthaquinone, 6- and 7- hydroxy-β-naphthaquinones[10]).

The infra-red spectrum of Fuerstiaquinone (Nujol, HCB, and CCl_4) showed two medium absorptions in the 3500-3000 $cm.^{-1}$ region, one at 3530 $cm.^{-1}$ and the other at 3350 $cm.^{-1}$. The absorption at 3350 $cm.^{-1}$ was attributed to the hydroxyl group which is strongly hydrogen-bonded with one of the quinone carbonyl groups (cf. Dimroth test and Karrer's isolation of cupric complex). In order to explain the absorption at 3530 $cm.^{-1}$ which is due to a free hydroxyl group, it is necessary to presume that, if a three oxygen chromophore ortho-hydroxyquinone system is present in Fuerstiaquinone, an equilibrium with respect to the hydrogen bonding (i.e., ) must exist. If this is not so, the only other reason for two absorptions is to be found in the presence of an imide or another hydroxyl group (and therefore no dicarbonyl system) in the Fuerstiaquinone molecule. An elementary analysis of Fuerstiaquinone proved the absence of nitrogen (On sending a sample of Fuerstiaquinone for nitrogen estimation, Drs.

Weiler and Strauss, Oxford, reported that nitrogen was found to be absent) and thus excluded the former. If the latter is the case then a three oxygen (i.e. hydroxyquinone) chromophore is impossible.

Although it has been observed by Macbeth et al.[5] that many hydroxylated α-naphthaquinones exhibit a very similar broad band in the visible region like β-naphthaquinones and the position and intensity of the maxima increase with the increasing number of substituents, it can be excluded from our consideration on the ground that the presence of only one or two hydroxyl groups does not increase the intensity as much as shown by Fuerstiaquinone (log ϵ 4.1). This is illustrated by the following four typical quinones.[11]

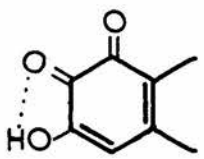
	$\lambda_{max.}$	log ϵ
5-hydroxy-1:4-naphthaquinone (Juglone)	425	3.6
2-hydroxy-1:4-naphthaquinone (Lawsone)	395	3.3
5:8-dihydroxy-1:4-naphthaquinone (Naphthazarin)	450 485	3.5 3.7
2:3-dihydroxy-1:4-naphthaquinone (Iso-naphthazarin)	445	3.2

It is noteworthy that the presence of three or more hydroxyl groups can only increase the intensity of absorption comparable to that of Fuerstiaquinone, but in that case the wavelength of the maximum is considerably higher than 435 mμ($\lambda_{max.}$ of Fuerstia-

:quinone). This is illustrated below:

	$\lambda_{max.}$	log ϵ
3:5:8-trihydroxy-2-methyl-1:4-naphtha-:quinone (Hydroxydroserone)[5]	488	3.83
2:5:8-trihydroxy-1:4-naphthaquinone (Naphthapurpurin)	485	3.85

Considering the facts described above, it was thought that Fuerstiaquinone might contain the chromophoric system (I) but this does not convincingly explain all properties (e.g. the infra-red OH absorption).

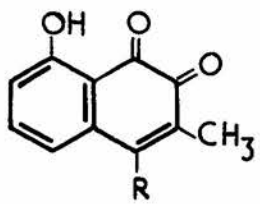


I

An analgous structure[10] has been proposed for Celastrol which has a similar ultra-violet and visible spectrum, indicating the presence of the same chromophoric system.

Celastrol is a ruby red pigment found in the outer bark of the root of Celastrus scandens.[12] An empirical molecular formula, $C_{22}H_{30}O_3$, was assigned to this compound by O. Gisvold who later published a series of papers[13] on the structure of this compound. From the results of degradative experiments and

colourimetric tests, he suggested the structure (II) for Celastrol which was also supported by the works (spectrophotometric analysis) of L.F. Fieser and R.N. Jones.[10]



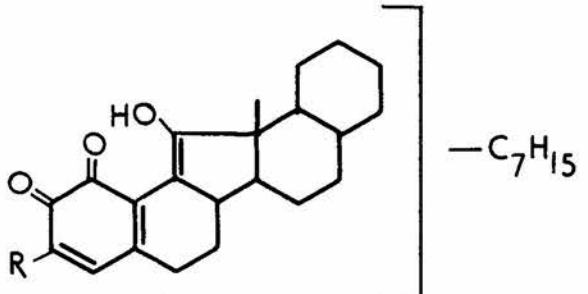
$R = C_{11}H_{23}$

II

In 1951 Bhatnagar and Divekar[14] isolated pristimerin, an orange crystalline compound, from Pristimera indica (Wild) and from P. grahami of the family Celastraceae. Kamat et al.[15] and Nakanishi et al.[16] assigned the empirical formula, $C_{30}H_{40}O_4$, to this compound. The similarity in the source and ultra-violet spectrum of pristimerin to those of celastrol demonstrated a close relationship to exist between the two substances. Investi-:gations by Nakanishi et al. have proved that pristimerin is identical with celastrol monomethyl ether (prepared by the action of diazo-methane on celastrol). In order to reconcile this fact, Gisvold's formula for celastrol had to be modified to $C_{29}H_{38}O_4$, which was found to be in better accord with analysis than the previously assigned one.

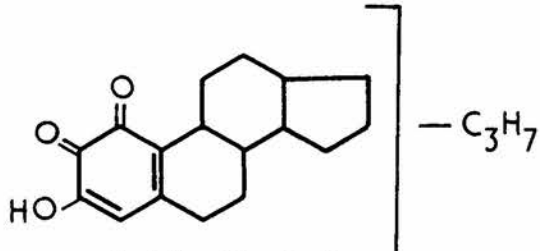
On studying the light absorption properties of pristimerin, the Japanese workers, Nakanishi, Kakisawa and Hirata fully agreed

with Gisvold on the point that pristimerin is an ortho-quinone (cf. Fig.I and II). These workers favoured an ortho-benzoquinone structure with an additional conjugation instead of the β -naphtha-:quinone structure as suggested by Gisvold. Based on the results of degradative experiments and on the isolation of a substituted picene, on distilling pristimerin with zinc dust, Nakanishi et al. proposed structure (III) for pristimerin and (IV) for celastrol.[16]



III (R = OCH_3)
IV (R = OH)

It should be remarked that in many chemical reactions,[16b] which will be described in more detail later in this thesis, Fuerstiaquinone has been found to behave in the same manner as pristimerin. More-:over, bearing the fact in mind that the plant Fuerstia africana possesses lactogenic property, one would be inclined to think that Fuerstiaquinone might be a new type of quinone having some sort of steroid structure. At first sight one feels that the structure (V) for Fuerstiaquinone is not an absolute improbability.



V ($C_{20}H_{26}O_3$)

A diagnostic test for ortho-quinones is the preparation of a quinoxaline derivative by condensing the quinone with o-phenylene-diamine. In the case of Fuerstiaquinone repeated attempts were unsuccessful.

If the three oxygen atoms are present in Fuerstiaquinone as shown in structure (V), one would expect two strong infra-red absorptions, one due to the free, and the other due to the hydrogen-bonded carbonyl group. In fact, the spectrum showed a strong absorption at 1590 cm.$^{-1}$ (Nujol) and only a very weak absorption at 1635 cm.$^{-1}$. No other absorptions were present between 1575 cm.$^{-1}$ and 1750 cm.$^{-1}$. Although a sufficient amount of infra-red data is not yet available in the quinone field for confident correlations to be attempted, none the less useful conclusions may be derived from the carbonyl stretching frequencies recorded in Table (I).

TABLE I.

Compound	C=O frequency(cm.$^{-1}$)	Compound	C=O frequency(cm.$^{-1}$)
Benzoquinone[17]	1660		
1:4-naphthaquinone[18]	1664	3:8-pyrenequinone	near 1640
Anthraquinone[17]	1676	3:10-pyrenequinone	near 1640
Anthrone	1653		
Oxanthrone	1676		

Josien and Fuson[19] confirmed the range 1680-1660 cm.$^{-1}$ for the carbonyl stretching frequencies of para-quinones and noted that those due to ortho-quinones were higher. Hädzi and Sheppard[20] state

that extended quinones have lower frequencies (1655-1635 cm.$^{-1}$).

As the spectrum of Fuerstiaquinone did not show any strong absorption between 1750 cm.$^{-1}$ and 1660 cm.$^{-1}$, it is evident from the above facts that an unassociated carbonyl group cannot be present in the molecule. This seems difficult to explain because Fuerstiaquinone cannot have two carbonyl groups both hydrogen-bonded which would imply the presence of four oxygen atoms in the molecule. The only plausible explanation that can be advanced is that Fuerstiaquinone contains one carbonyl and two hydroxyl groups. The two sharp absorption bands of medium intensity at 3530 cm.$^{-1}$ and at 3350 cm.$^{-1}$ (with almost equal peak heights)are, therefore, not due to a single hydroxyl group present in a hydrogen-bonding equilibrium, as previously discussed. It is, of course, admitted that intermolecular hydrogen bonds, in which no resonance structures are involved, can give rise to strong absorption bands in the region where Fuerstiaquinone has been found to absorb, but in that case the intensity of the band would have been considerably greater and the shape of the band would have been broad unless a specific dimer is formed. The broadness in shape is attributed to the fact that the OH group associates into various polymeric forms in which the molecules are involved in hydrogen bonding to different extents. In single bridge dimeric alcohols, which are unable to associate into polymeric forms[21] due to steric hindrance, only weak hydrogen bonds are involved and these usually absorb (sharp bands) near 3500 cm.$^{-1}$. In contrast to this, normal alcohols, in which extensive polymerisation occurs

absorb (broad band) near the range 3400-3200 cm.$^{-1}$. Kuhn[22] examined a number of compounds and found the absorption of dimeric alcohols in the range 3525-3472 cm.$^{-1}$ and that from the polymeric materials in the range 3341-3338 cm.$^{-1}$.

As the intermolecular hydrogen bonds in single bridge or polymeric compounds are not stabilised by resonance, they are readily broken on dilution. A 1% solution of Fuerstiaquinone (freshly prepared and thrice recrystallised from iso-octane) in carbon tetrachloride was diluted to give solutions of several concentrations and the spectrum of each sample (1%, ½%, ¼%, ⅛% $\frac{1}{16}$% etc.) was determined using a lithium fluoride prism which gives greater resolution. In all cases two strong, sharp absorptions were found at 3480 cm.$^{-1}$ and at 3306 cm.$^{-1}$. The relative heights of the peaks remained accurately constant. This suggests that these two absorption peaks are not due to dimerisation or polymerisation and hence must involve the presence of two hydroxyl groups in Fuerstiaquinone. It was decided that most probably the absorptions at 3306 cm.$^{-1}$ and at 3480 cm.$^{-1}$ represented a bonded and a non-bonded hydroxyl group respectively. These latter values, determined using the lithium fluoride prism, are more accurate. Those using sodium chloride are recorded, as this is the prism normally used by workers at large. In order to confirm the presence of two hydroxyl groups in Fuerstiaquinone, attempts were made to prepare methyl or acetyl derivative of Fuerstiaquinone.

Brockmann's observation in the case of hypericin and similar

compounds is that ketene acetylates only the free hydroxyl groups leaving unreacted, the chelated ones.[23] This led us to attempt an acetylation of Fuerstiaquinone with ketene in the hope of getting a monoacetyl derivative which would still contain a bonded hydroxyl group and would, therefore, show its characteristic absorption in the infra-red.

On passing ketene gas (obtained by cracking acetic anhydride at 400°C) through a solution of Fuerstiaquinone in ligroin, a deeper red solution was obtained. This, after freeing from excess ketene[24] followed by chromatography, afforded a brown oil which could not be crystallised. The oil was examined by infra-red and the spectrum showed no absorptions due to hydroxyl groups.

In the case of Brockmann's compound the hydroxyl groups were in the peri-position of the quinone carbonyl and therefore could form a more stable six-membered chelate than a five membered one as present in Fuerstiaquinone. Perhaps this might be the reason for acetylation of both the hydroxyl groups of Fuerstiaquinone.

Acetylation of Fuerstiaquinone with acetic anhydride in presence of pyridine at room temperature gave a brownish yellow crude amorphous powder (m.p. 75-85°). The product could not be crystallised as it was very soluble in all common organic solvents except ligroin in which it was completely insoluble. Attempted crystallisations from benzene-ligroin or ethanol-ligroin gave a

yellow oil which on keeping exposed to air turned into a dark oil.

When a solution of Fuerstiaquinone in acetic anhydride containing little pyridine was refluxed for ca. two hours, a dark brown solution was obtained. The colour was probably due to the rearranged product, and on working the solution, a dark coloured amorphous solid was obtained which could not be crys-:tallised.

Acetylation of Fuerstiaquinone under Thiele condition yielded a buff coloured amorphous powder (m.p. 65-69°) which, being easily soluble in all common organic solvents at room temperature, could not be crystallised. However, it was noted that the substance, on treatment with ligroin, left a very small amount of insoluble material which crystallised from aqueous ethanol in the form of long colourless needles. The extremely poor yield of the crystals (i.e., few needles from 250 mg. of Fuerstiaquinone) did not permit further investigation.

Attention was next directed towards making the methyl der-:ivatives. Methylation methods, which involve the use of (1) dimethyl sulphate and sodium hydroxide or (2) methyl iodide and silver oxide could not be applied as the first would cause re-:arrangement and the second, oxidation. Therefore, recourse was taken to use diazo-methane as the methylating agent.

On mixing ethereal solutions of Fuerstiaquinone and diazo-:methane, the red colour of the former immediately turned very dark and no nitrogen was evolved. The reaction product was ob-

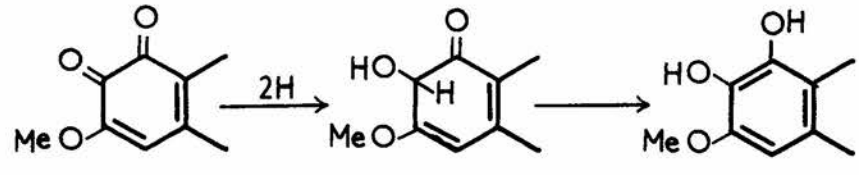
:tained as a dark brown oil which did not give any solid material after purification by chromatography. When the mixture of diazo-:methane and Fuerstiaquinone was allowed to stand for 2-3 days, the dark colour of the solution gradually changed to dark brown, yellowish brown, yellowish dark, greenish dark and finally in about a week's time became sherry-red colour.

It is known that diphenyl diazomethane reacts with o-quinones with evolution of nitrogen and formation of methylene ether deri-:vatives of the corresponding hydroquinones. As nitrogen was not evolved in the case of Fuerstiaquinone, the methyl or methylene ether was not formed; presumably an addition reaction took place (cf. reaction of diazomethane with ∝-naphthaquinone to yield lin-naphthindazole-4, 9-quinone[25]).

Later, methylation was attempted with methyl iodide and fused potassium carbonate. On refluxing a solution of Fuerstia-:quinone in anhydrous acetone with the reagents mentioned for about two hours, the deep red colour of the solution changed into dark reddish brown. On removing the solvent, the crude reaction product was obtained as a dark oily semi-solid mass which did not crystallise. Purification by chromatography afforded only a dark, slightly red amorphous powder, m.p. 43-50°. (Found: C, 73.06; H, 8.30; OCH_3, 12.26%. $C_{20}H_{24}O(OCH_3)_2$ requires C, 77.01; H, 8.77; OCH_3, 18.1%. $C_{20}H_{25}O_2(OCH_3)$ requires C, 77.30; H, 7.97; OCH_3, 9.45%). The infra-red spectrum (CCl_4) did not show any OH-absorption.

The failure of repeated attempts to obtain a pure methyl or acetyl derivative of Fuerstiaquinone under different conditions of the reactions just described, presented a very baffling sit-:uation.

Nakanishi *et al.*[16] observed that pristimerin, on reduction with potassium borohydride or hydrogen in presence of platinum, was converted into a colourless derivative, pristimerol ($C_{30}H_{42}O_4$) which contained two hydrogen atoms more than pristimerin. In pristimerol, the original methoxyl group was retained, and two hydroxyl groups had been formed as evidenced by formation of a di-*p*-nitrobenzoate and a dimethyl ether. The infra-red spectrum of pristimerol showed that a hydroxylated benzenoid moiety was present in the compound and the formation of the latter was presumed to proceed through the following sequences, in which only the carbonyl group at position two was reduced since the conjugated system incorporating the other carbonyl group, $-CO-C=C-C=C-OCH_3$, can be regarded as a carbomethoxy vinylog.



If Fuerstiaquinone has the part structure (I) then, on reduction

with potassium borohydride or with hydrogen in presence of platinum, it should similarly yield a colourless dihydro derivative which will presumably contain the pyrogallol system.

Fuerstiaquinone was reduced with potassium borohydride under exactly the same condition as used in the case of pristi-:merin. A solution in ethanol was treated with potassium boro-:hydride and the colour of the quinone disappeared completely within a few seconds. The excess borohydride was decomposed with acetic acid. On adding water to the mixture a milky white precipitate was obtained which, on contact with air, very quickly (about a minute) darkened to a chocolate brown colour. The same observation was recorded when acetic acid was not used.

The reduction product was found to be more susceptible to aerial oxidation when in the solid state rather than in solution; especially when the solid was heated for crystallisation, an intense dark colour was obtained due to very rapid oxidation. It was evident, therefore, that the reduction product of Fuerstia-:quinone was less stable than that of pristimerin. The borohy-:dride reduction was carried out in different conditions and attempts to crystallise the product in the form of a white solid by working in an inert atmosphere, were unsuccessful. In every case a more or less intense chocolate-brown coloured amorphous powder was obtained which melted within a wide range of temper-:ature, indicating that the substance was impure.

The isolation of a product resulting from the reduction of

Fuerstiaquinone with hydrogen in presence of platinum catalyst, was also unsuccessful. Upon hydrogenation, the ethanolic solution of the quinone went completely colourless, but the reaction product, on coming in contact with air, rapidly (ca. 5 minutes) oxidised back to the quinone.

The strikingly great instability of the reduction product of Fuerstiaquinone compared to pristimerol towards oxidation, must be due to different structural features connected with the quinonoid system of the two compounds. These will be discussed later as these have no immediate bearing on the account of the oxygen functions of Fuerstiaquinone which is the subject of discussion at the moment.

Attempts were next made to acetylate or methylate the reduction product of Fuerstiaquinone. Reductive acetylation with zinc dust and acetic anhydride in presence of a small amount of triethylamine yielded an almost white amorphous powder (m.p. 94-98°), which could not be crystallised. Analysis proved the substance to be a diacetate (Found: C, 71.73; H, 7.72; OAc 19.4%. $C_{20}H_{24}O(OAc)_2$ requires C, 72.36; H, 7.53; OAc, 21.6%). The infra-red spectrum (nujol) of the compound showed strong absorptions at 1778 cm.$^{-1}$ and 1750 cm.$^{-1}$ and weak ones at 1637 cm.$^{-1}$, 1616 cm.$^{-1}$ and 1568 cm.$^{-1}$. A broad medium band extending from 3750 cm.$^{-1}$ to 3150 cm.$^{-1}$ and having maximum at 3500 cm.$^{-1}$ was also present. The absence of any absorption at 1590 cm.$^{-1}$ should be noted. The reductive diacetate, on keeping for a long time, gradually changed

its colour from very pale yellowish white to yellow, then yellowish brown and finally yellowish orange.

On investigating the carbonyl frequencies of over a hundred sterol acetates, propionates, etc., Jones and co-workers[26] have found all the saturated materials to absorb in the range 1742-1735 cm.$^{-1}$. According to Bellamy,[27] the carbonyl stretching frequencies in the case of normal saturated esters lie within the range 1750-1735 cm.$^{-1}$. In contrast to this, the vinyl esters, which possess the structure CO-O-C=C absorb at markedly higher frequencies, regardless of whether the double band is normal or part of an aromatic ring. For example, vinyl acetate absorbs at 1776 cm.$^{-1}$. The normal range of carbonyl absorption in the case of vinyl esters is given by Bellamy[27] as 1770-1745 cm.$^{-1}$.

It is clear that the absorptions at 1750 cm.$^{-1}$ and at 1778 cm.$^{-1}$ in the spectrum of Fuerstiaquinone reductive diacetate must have arisen from the normal and vinyl ester carbonyls. In other words, the reduction product of Fuerstiaquinone must contain an enolic as well as an alcoholic hydroxyl group. The broad absorption band (3750-3150 cm.$^{-1}$) is due to a hydroxyl group, probably chelated with the ester carbonyl. For comparison salicylaldehyde and ortho-hydroxy acetophenone[28] exhibit broad but weak OH absorption bands extending from 3500 cm.$^{-1}$ to beyond 2900 cm.$^{-1}$. However, this can not be reconciled with the fact that any additional band due to the chelated carbonyl of the ester is absent in the infra-red spectrum. The strong chelation

of the hydroxyl group is presumably responsible for its resistance to acetylation. The change in the colour (e.g., from very pale yellowish white to deep yellowish orange) of the reductive diacetate in the solid state is probably due to the aerial oxidation of the hydroxyl to a carbonyl group with the introduction of a quinonoid system in the molecule. This will be dealt with later. Further acetylation of the reductive diacetate was attempted with acetic anhydride and pyridine but only a dark brown amorphous solid was obtained as in the case of regular acetylation of Fuerstiaquinone with acetic anhydride and pyridine.

A solution of Fuerstiaquinone in absolute ethanol was reduced with hydrogen in presence of Adam's catalyst and the colourless solution was treated with acetic anhydride. The mixture, on standing overnight in absence of oxygen, did not produce the desired derivative. During isolation, the product became more and more intensely coloured towards red on coming in contact with air and ultimately a deep red amorphous solid, probably Fuerstiaquinone, was obtained.

While methylation or acetylation under a variety of conditions did not afford a crystalline product, the reductive acetylation with acetic anhydride in presence of pyridine proceeded smoothly to give a colourless, crystalline and optically active dihydrotriacetyl derivative (m.p. 193-194°; Found: C, 70.57; H. 7.77; OAc, 35.6%. $C_{20}H_{25}(OAc)_3$ requires C, 70.56;

H, 7.74; OAc, 29.1%). The ultra-violet spectrum of the reductive triacetate (Fig.III) showed a maximum at 292 mμ (log ε 2.8) and a shoulder-like peak at 282 mμ (log ε 2.7) which could only be attributed to a benzenoid nucleus. The infra-red spectrum (HCB) exhibited two strong, very sharp absorptions at 1750 cm.$^{-1}$ and 1774 cm.$^{-1}$ (cf. diacetate) arising from the stretching vibrations of the normal and vinyl ester carbonyl groups respectively. This indicated that the reduction product must contain an enolic as well as an alcoholic hydroxyl group. No OH absorption between 3700-3000 cm.$^{-1}$ was present. In the 1700-1575 cm.$^{-1}$ region only two absorption peaks were found to be present, e.g. one strong absorption at 1617 cm.$^{-1}$ and a weak one at 1638 cm.$^{-1}$. The absorption at 1617 cm.$^{-1}$ was due to the conjugated C=C system present in the hexachlorobutadiene used as mulling agent and that at 1638 cm.$^{-1}$ probably arose from an isolated double bond between two carbon atoms present in the reductive acetate. The disappearance of the absorption peak at 1590 cm.$^{-1}$ (cf. spectrum of Fuerstiaquinone) suggested once again that it was probably related with the quinone carbonyl. Determination of the infra-red spectrum using the high dispersion of a lithium fluoride prism showed two sharp absorption peaks at 3066 cm.$^{-1}$ and at 3023 cm.$^{-1}$ which definitely proved the presence of C-H groups forming part of an aromatic ring[29] in the reductive triacetate.

As will be remembered, from the fact that the infra-red

spectrum of Fuerstiaquinone does not show any normal carbonyl absorption, it was concluded before that Fuerstiaquinone contains one carbonyl (bonded) and two hydroxyl groups. Now it is clear that one of these hydroxyl groups is alcoholic (absorption peaks at 1750 $cm.^{-1}$ in the spectra of the reductive di- and triacetates).

It is known that quinone systems, on reduction revert to the less energy containing and, therefore, more stable aromatic systems. In Fuerstiaquinone if both the hydroxyl groups are substituted in the quinone ring then, on reduction, a trihydric phenol will be produced. But as an alcoholic hydroxyl group is present in the reduction product of Fuerstiaquinone, the quinone ring cannot contain more than one hydroxyl group. It may be possible that the quinone ring does not contain any hydroxyl group at all because the chelation of the quinone carbonyl with the hydroxyl group does not demand the position of the latter in the same ring. But the fact that the reduction product of Fuerstiaquinone (Pt/H_2) produced a green colouration on treatment with $FeCl_3$, suggested that the former possessed an o-dihydroxy benzene system (also compare the doublet in the ultra-violet spectrum of the reductive triacetate with that in the spectrum of catechol dimethyl ether[30]). Therefore, the quinone ring must contain a hydroxyl group substituted at the ortho position with respect to the carbonyl.

It was considered at this stage that the presence of two

types of hydroxyl groups (i.e. alcoholic and phenolic) in the reduction product of Fuerstiaquinone, might be demonstrated by methylating the same with diazomethane and thereby obtaining a dimethyl derivative that would still contain a free alcoholic hydroxyl group. Accordingly, Fuerstiaquinone was reduced with hydrogen in presence of platinum catalyst and the reduction product was treated with diazomethane. The reaction product was obtained as a very viscous brown oil which, on purification by chromatography, afforded a pale yellowish white amorphous solid that could not be crystallised. Analysis of the crude substance (m.p. 65-68°) gave result corresponding to the dimethyl derivative. (Found: OCH_3, 16.28%. $C_{20}H_{26}O(OCH_3)_2$ requires: OCH_3, 18.02%). Infra-red spectrum (nujol) of the compound showed a strong, broad absorption band with maximum at 3341 $cm.^{-1}$ which was presumably due to the free alcoholic hydroxyl group present in the polymeric form.

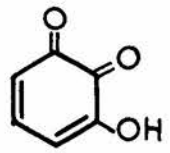
Confirmation of the presence of two hydroxyl groups in Fuerstiaquinone was obtained from the infra-red spectrum of deuterated Fuerstiaquinone. The infra-red spectrum (CCl_4) of Fuerstiaquinone was determined in the 3700-3200 $cm.^{-1}$ region using a lithium fluoride prism. The OH absorptions appeared at 3480 $cm.^{-1}$ and 33 06 $cm.^{-1}$ with peak heights of 3.3 and 3.65 inches respectively. Fuerstiaquinone, present in the same solution that was used for spectrum determination, was then deuterated. The carbon tetrachloride solution containing the deuterated

material was completely freed from moisture and brought to the original concentration. The infra-red spectrum of this solution was determined using lithium fluoride prism and the same cell thickness as before. The spectrum showed no absorption peak in the range 3600-3200 cm.$^{-1}$, and instead two new peaks appeared at 3008 cm.$^{-1}$ and 2846 cm.$^{-1}$. The disappearance of both the peaks at 3480 cm.$^{-1}$ and 3306 cm.$^{-1}$ in the spectrum of deuterated Fuerstiaquinone and at the same time the appearance of two new peaks proved that both the absorption peaks were due to two hydroxyl groups. Further, analysis showed that Fuerstiaquinone contained 1.5 active hydrogen atoms (Zerewitinoff).

As it is now proved beyond any doubt that the Fuerstiaquinone molecule bears two hydroxyl groups, there must be one and only one carbonyl group to account for the three oxygen atoms. In other words Fuerstiaquinone must be a methylene quinone. Therefore, the earlier chromophore (I), derived from the similarity in the ultra-violet spectra of Fuerstiaquinone and celastrol, was abandoned.

Fuerstiaquinone did not respond to the ethylene diamine test[31] for o-benzo- and α- or β-naphthaquinones, a fact which also indicated its methylene quinone structure. It is of interest to note that Willstätter et al.[32] prepared 3-hydroxy-1:2-benzoquinone (VI), and found that the substance was colourless. The compound also did not give ordinary quinone reactions. Although the structure of the compound prepared by these workers

can be doubted for the reason that o-benzoquinone is red, none the less it may bear some significance.



VI

Another fact that may be pointed out is that the methylene quinones can be regarded as dihydroaromatic analogues of alde-:hydes and resemble the latter in several properties, one of which is the polymerisability. The great tendency of Fuerstia-:quinone to polymerise in presence of resins might be due to this structural feature.

Flett[17] has shown that an α-hydroxy group, which can form a six-membered chelate ring with the quinone carbonyl, invariably causes the appearance of a low carbonyl frequency in the range 1640-1590 cm.$^{-1}$, to be compared with the range 1680-1660 cm.$^{-1}$ in the case of unsubstituted p-quinones. In the case of the chelated carbonyls in o-quinones, the range will, of course, be higher. Although the absorption frequency of Fuerstiaquinone is at the bottom end of the range quoted, i.e. at 1590 cm.$^{-1}$, it must represent the carbonyl group chelated with the hydroxyl in the ortho-position.

It was intended, at this stage, to determine the carbonyl

frequencies of methylene quinones. Methylene anthrone[33] was prepared by condensing anthrone with formaldehyde in presence of piperidine. Infra-red spectrum (nujol) of the substance showed strong absorption at 1636 cm.$^{-1}$ arising from the C=O stretching vibration. Preparation of benzylidene anthrone[34] was attempted from anthrone and benzaldehyde under the same conditions as used in the case of methylene anthrone; but this was unsuccessful.

The infra-red spectra of celastrol (kindly supplied by Prof. O. Gisvold, University of Minnesota) was determined for comparative study with that of Fuerstiaquinone. The spectrum (nujol) showed strong absorptions at 1595 cm.$^{-1}$ and at 1710 cm.$^{-1}$ and a very weak absorption at 1642 cm.$^{-1}$. No other absorptions between 1750-1550 cm.$^{-1}$ were present. In the hydroxyl region the spectra of celastrol (in CCl_4 or HCB) exhibited two medium absorptions at 3290 cm.$^{-1}$ and 3165 cm.$^{-1}$.

From the spectral data, one is forced to believe that the structure (IV) proposed for celastrol by Nakanishi *et al.* is not fully correct. Such a structure in which both the carbonyl groups are hydrogen bonded cannot give rise to a strong absorption peak at 1710 cm.$^{-1}$. The frequency, 1710 cm.$^{-1}$, is too high even for a free quinone carbonyl group. It seems that the oxygen function contained in the five-membered ring is possibly present in the keto rather than the enol form, and this causes the absorption at 1710 cm.$^{-1}$. For comparison,

cyclopentanone absorbs at 1740 cm.$^{-1}$ and cyclohexanone at 1710 cm.$^{-1}$. Now, if the oxygen function in the five-membered ring is present in the keto form, then one of the quinone carbonyls would be free and, therefore, the spectrum of celastrol should show a normal carbonyl absorption. But this is not the case. The only carbonyl absorption at 1595 cm.$^{-1}$ (besides the 1710 cm.$^{-1}$ one) suggests that celastrol may be a methylene quinone like Fuerstiaquinone.

Having been established that Fuerstiaquinone is a methylene quinone, the next point that calls for decision is whether it is of an ortho- or a para-structure. That Fuerstiaquinone possesses the para-methylene quinone structure was inferred from the following facts:

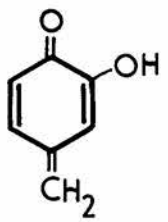
(1) In contrast to the o-methylene quinones, the p-compounds are highly reactive and very readily undergo molecular rearrangement.[35]

(2) Almost all the methylene quinones occurring in nature are of the para-type.

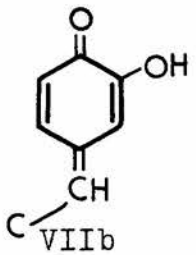
(3) The formation of a degradation product, which will be described later, cannot be explained by assuming the o-methylene quinone structure.

Being a para-methylene quinone, Fuerstiaquinone may have

any of the following two structural types:



VIIa

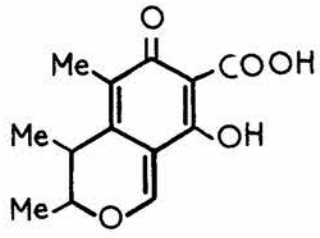


VIIb

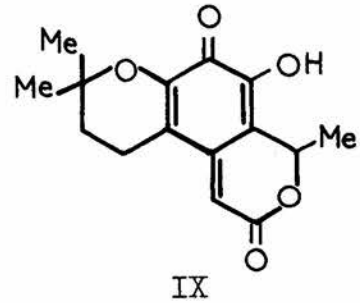
But, as Fuerstiaquinone did not produce formaldehyde on ozonolysis, the structure (VIIa) is not possible.

It has already been mentioned that the absorption maximum in the visible spectrum of Fuerstiaquinone is at 435 mμ (log ε 4.1). This is an appreciably high wavelength for simple p-methylene quinones. For example, citrinin[36] (VIII) absorbs at 333 mμ (log ε 3.92) and fuscin[37] (IX) at 355 mμ (log ε 4.44). Cooke et al.[9] has suggested that the αβ-unsaturation expresses itself in both the ortho- and para-quinones in two main effects, viz., (a) the appearance of an inflexion instead of the band located at ca. 330 mμ which is characteristic of the carbonyl group in the system C=C-C=O and (b) the displacement of the long wave length band to a region of still greater wave length (cf. spectrum of β-methyl pyrano-1:2-naphthaquinone[9]). It is, therefore, necessary to extend the chromophore of Fuerstiaquinone (VIIb) by additional

conjugations.

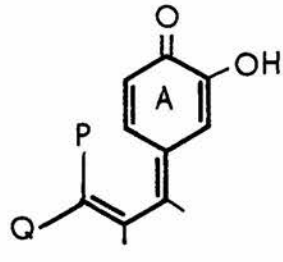


VIII

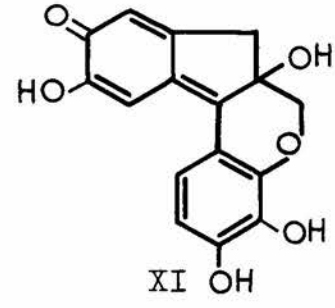


IX

On the basis of the formula, $C_{20}H_{26}O_3$, and a single carbonyl group, Fuerstiaquinone molecule can have 1, 2 or 3 rings con-:taining 6, 5 or 4 double bonds respectively. For reasons which will be presented later, Fuerstiaquinone must contain at least three rings. Therefore, the molecule cannot have more than four double bonds; or, in other words, the chromophore (VIIb) can be extended by only one double bond. Accordingly the chromophore (X) is being proposed for Fuerstiaquinone. It is possible for this chromophore to absorb maximally at 435 mμ. For comparison, Haematein (XI) absorbs at 430 mμ (logϵ 4.60).[38]



X



XI

Most of the known methylene quinones contain an oxygen

function at the end of the conjugated system remote from the diunsaturated carbonyl group, e.g., fuscin (IX) contains another carbonyl group, and citrinin (VIII), purpurogenone[39](XII), haematein[40](XI) and the closely related brazilein contain a cyclic ethereal oxygen atom. This feature isolates the chromophore from labile hydrogen atoms and prevents a tautomeric rearrangement of the colouring matter to a phenol. In Fuerstiaquinone, the carbon atom (9) terminating the chromophoric system should not, therefore, support a carbon atom with directly linked hydrogen; in other words, if P and Q are carbon atoms then they should not bear any hydrogen atom.

XII

It is interesting to note that haematein shows infra-red absorption at 1595 cm.$^{-1}$ due to the carbonyl which is hydrogen bonded to the ortho-hydroxy group. This strongly supports the previous assignment of the absorption band at 1590 cm.$^{-1}$ to the carbonyl group of Fuerstiaquinone. However, it must be mentioned that attempts to prepare a carbonyl derivative of Fuerstiaquinone, e.g., an oxime or 2:4-dinitrophenylhydrazone,were not successful.

When the chromophore became clear, the next thing was to determine the basic carbon skeleton of Fuerstiaquinone. In the quinone field, the generally adopted method for this is the zinc dust distillation (cf. Hypericin,[23] Aphins,[41] Cochineal,[42] Alizarin etc.). Moreover, considering the formation of picene from pristimerin under the same condition, it was strongly believed that Fuerstiaquinone should also yield a somewhat similar type of hydrocarbon on distillation with zinc dust.

Zinc dust distillation of Fuerstiaquinone in the semi-micro scale was found to proceed with the charring of a large amount of the pigment and therefore the product was obtained in extremely poor yield. In order to avoid this, several micro zinc dust distillations of Fuerstiaquinone were carried out. The crude distillate, on chromatographic separation, yielded four different substances. The chief product was a colourless oil (146 mg. from 1.35 g. of Fuerstiaquinone) having a kerosine-like smell. It was insoluble in polar and soluble in non-polar solvents. Its solution fluoresced purple in the ultra-violet light. The oil partly crystallised in the form of long colourless plates but the latter could never be freed from the contaminating oil. Repeated chromatography with the oil failed to give a separation and it seemed that the oil was probably a mixture of stereoisomers. The crystalline solid was found to be low melting, the melting point probably lying between 40-50°. The oil mixture did not form a picrate or a 2:4:7-trinitro-fluorenone complex. Ultra-

:violet spectrum (Fig.IV) of the oil showed maxima at 228 and 273 mμ which could only be attributed to a benzene ring present in the molecule. However, from the properties mentioned, it was considered that this distillation product was probably partly aromatic and therefore contained a larger proportion of hydrogen than expected. In other words, zinc dust had not effected complete dehydrogenation of the Fuerstiaquinone molecule. It is apparent that the benzene ring was formed by aromatisation of the ring A (X).

Among other products of the zinc dust distillation, one was an orange coloured amorphous solid whose solution fluoresced green in the ordinary light. The ultra-violet spectrum of this substance (Fig. V) could not be identified with that of any known aromatic hydrocarbon. It seemed that the compound might be of the 3:4-benztetraphene[43] type, probably formed by a dimer-:isation process during heating. The compound did not form a picrate or a trinitrofluorenone complex.

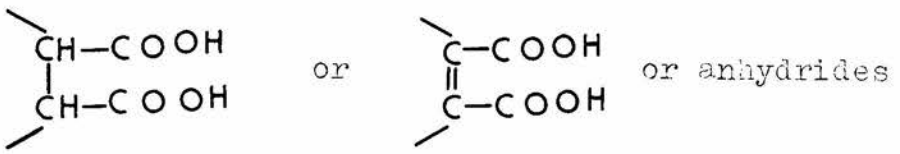
Two other solids (colourless, crystalline plates, m.p. 103° and 215-18°) were also obtained as the distillation products; but their yields were extremely poor. The ultra-violet spectrum of the solid (m.p. 103°) showed maxima at 225-230 and 255-260 mμ (Fig.VI) which could only be attributed to a simple benzene derivative. The other solid was found to sublime at atmospheric pressure. Its ultra-violet spectrum could not be determined. In order to obtain these two compounds in sufficient amount, the zinc

dust distillation was repeated with a greater quantity of Fuerstiaquinone, but the same products were not obtained again (For detailed discussion see 'Experimental' section).

The zinc dust distillation did not provide any useful information regarding the basic carbon skeleton of Fuerstiaquinone. Oxidative degradations were then carried out in an endeavour to obtain recognisable fragments. At first Fuerstiaquinone was reacted with alkaline hydrogen peroxide at room temperature. The oxidation products were separable by paper chromatography, which demonstrated the presence of at least three acidic substances. One of these crystallised from the oxidation mixture on acidification in the form of long, colourless hexagonal prisms (m.p. 222-3°). This substance was of great help in subsequent studies as it offered valuable information regarding the structure of Fuerstiaquinone. The other two acids were separated on a cellulose column by elution with the same solvent mixture as employed in the original paper chromatography; they could not be crystallised. It was thought that the oxidation products as their methyl esters might separate more efficiently on alumina. The oxidation product was methylated with diazomethane and the mixture of esters was subjected to distillation and sublimation under reduced pressure before resorting to final chromatography. By doing this, four esters were separated suggesting that the oxidation product was actually a mixture of four acids, two of which had not been originally resolved. All

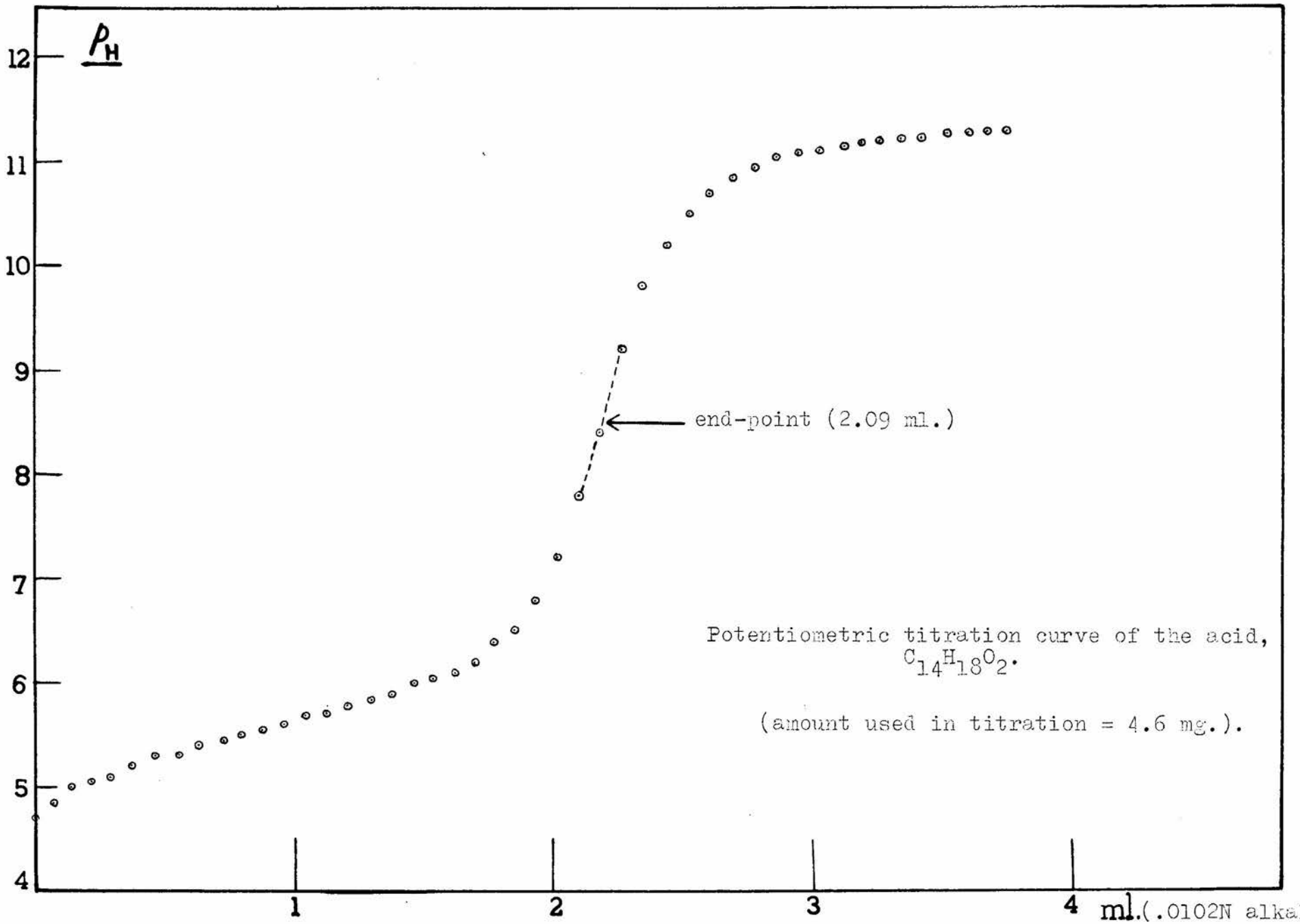
the individual esters were studied. The result of this will be described later.

The acid which had m.p. 222-3°, neither coupled with diazotised sulphanilic acid nor produced any colouration with $FeCl_3$ indicating the probable absence of a phenolic hydroxyl group in the molecule. The compound did not respond to the Fluorescein test which indicates that it could not contain the anhydride or dicarboxylic structure:



Infra-red spectrum (nujol) of the substance exhibited a strong peak at 1675 $cm.^{-1}$ and a weak one at 2654 $cm.^{-1}$ arising from the C=O and OH stretching vibrations[44] of the carboxyl group respectively. Other characteristic carboxylic acid absorptions, e.g. single bands at 1411 $cm.^{-1}$ and at 943 $cm.^{-1}$ (OH deformation vibration) and doublets between 1250-1280 $cm.^{-1}$ and between 1280-1310 $cm.^{-1}$, were also present. No ordinary OH absorption was found between 3000-3650 $cm.^{-1}$. The absence of any absorption peak between 1700-1875 $cm.^{-1}$ suggested that the substance did not possess an anhydride group.[45]

Combustion analysis of the acid gave figures which corres-



Potentiometric titration curve of the acid, $C_{14}H_{18}O_2$.

(amount used in titration = 4.6 mg.).

:ponded to the empirical formula, $C_{14}H_{18}O_2$ (Found: C,77.03, 76.97; H, 8.01, 8.28. $C_{14}H_{18}O_2$ requires C, 77.03; H, 8.31). On potentio-:metric titration, a curve was obtained whose shape indicated the presence of only one titratable group in the molecule, i.e. the acid is monobasic as a C_{28} dibasic acid is unlikely to have been formed from a C_{20} compound. From the end point, the molecular weight was calculated as 215 (Mol.wt. of $C_{14}H_{18}O_2$ is 218).

On treatment with diazomethane, the acid was converted into its methyl ester which crystallised in the form of thin colourless plates (m.p. 81°). Analysis of this agreed with the formula $C_{15}H_{20}O_2$. Found: C, 77.35; H, 8.38. Calc. for $C_{15}H_{20}O_2$: C,77.58; H, 8.62). The infra-red spectrum (nujol) of the sub-:stance also showed strong absorption at 1711 cm.$^{-1}$ due to the stretching vibration of the ester carbonyl. The bands at 1675 cm.$^{-1}$ and at 2654 cm.$^{-1}$ disappeared.

Solutions of the acid and its ester were found to fluoresce in the ultra-violet light. The ultra-violet spectrum (Fig.X) of the acid showed maxima at 280 mμ ($\log \epsilon$ 2.9) and 236 mμ ($\log \epsilon$ 3.9) which are characteristic of a simple benzene derivative. A benzene ring is evidently present in the compound and this view is confirmed by the characteristic absorption bands in infra-red spectrum of the acid at 1601 cm.$^{-1}$ (aromatic C=C) and at 3085 cm.$^{-1}$ (aromatic C-H). The band corresponding to the former appeared at 1592 cm.$^{-1}$ in the spectrum of the methyl ester.

It is known that benzene absorbs ultra-violet light maximally at 255 mμ (log ε 2.3) and at 200 mμ (log ε 3.6). With the introduction of simple alkyl substituents in the ring, the ultra-violet curve displaces slightly to longer wavelength and the intensity may also increase but to a small degree. The large bathochromic shift of the maxima as well as a considerable rise in their intensity as found in the ultra-violet spectrum suggested that the acid, $C_{14}H_{18}O_2$, probably contained a benzene ring with a carboxyl group (i.e. benzoic acid type). For comparison, benzoic acid, in ethanolic solution, exhibits maxima at 271 mμ (log ε 2.9) and at 226 mμ (log ε 4.0). The bathochromic shift of 9-10 mμ in the spectrum of the acid, $C_{14}H_{18}O_2$ can, therefore, be attributed to the alkyl substituents.[46]

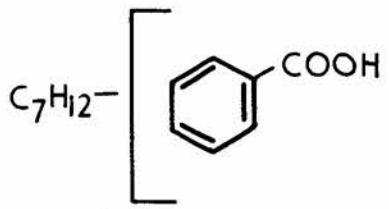
Further evidence bearing on this point was obtained from infra-red studies. It is known that an aromatic compound shows absorption band near 1600 cm.$^{-1}$ but, if examined carefully, one would find that a second band near 1580 cm.$^{-1}$ is also present in the form of a weak shoulder adjoining the main 1600 cm.$^{-1}$ band. In some cases it is so weak as to be practically undetectable. When a carbonyl group or any unsaturated group is directly attached to an aromatic ring, however, the intensity of this second 1580 cm.$^{-1}$ band is very considerably enhanced, and it becomes much more prominent. Accordingly it has been suggested by Randall *et al.*[47] that a band in the range 1575-1587 cm.$^{-1}$ should be regarded as a positive indication of conjugation of a

double bond with the aromatic ring. Rasmussen et al.[48] have made the more limited suggestion that absorption at 1600 cm.$^{-1}$ and at 1585 cm.$^{-1}$ may be indicative of the presence of a benzoyl group. The infra-red spectrum of the acid, $C_{14}H_{18}O_2$, exhibited a readily recognisable band of medium intensity at 1570 cm.$^{-1}$ in addition to a fairly strong one at 1601 cm.$^{-1}$. It was therefore concluded that the aromatic ring present in the compound was conjugated with a double bond or carbonyl group.

The carboxylic acid, $C_{14}H_{18}O_2$, is derived from the hydrocarbon, $C_{13}H_{18}$. If the latter possesses a benzene ring then, consideration of its molecular formula suggests that it should contain either one additional double bond or another ring. The presence of an additional double bond seems very unlikely since the acid is a product obtained from an oxidation reaction.

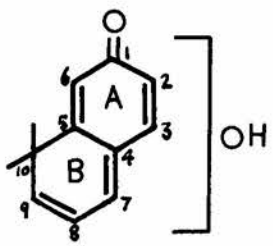
Moreover, if a double bond, conjugated with the benzene ring (as required by the infra-red characteristics), is present one would expect the acid to show a styrene type of absorption in the ultra-violet. Styrene shows ultra-violet maxima at 244 mμ ($\log \epsilon$ 4.1) and at 282 mμ ($\log \epsilon$ 2.7)[49] which are 8 and 2 mμ higher wavelength values than the corresponding maxima of the acid. Substitution will shift the maxima to still higher wavelengths. Therefore, the benzene ring is conjugated not with a double bond but with a carbonyl group. This evidently implies that a benzoic acid type structure is present in the acid in

question. This can be written as:-



It is noteworthy that, like benzoic acid, the compound, $C_{14}H_{18}O_2$, sublimes under normal atmospheric pressure (at about 178°C).

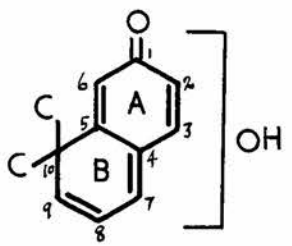
The formation of the acid, $C_{14}H_{18}O_2$, by the oxidative degradation of Fuerstiaquinone necessitates the introduction of another ring (B) in the molecule of Fuerstiaquinone. This is shown below:-



XIII

The hydroxyl group may be present at the 2 or 6 position. From the above structure, it is understandable that the benzene ring in the acid, $C_{14}H_{18}O_2$ must have been formed by the aromatisation of the ring (B). It cannot be formed by the aromatisation of the ring (A) because in that case it should have contained hydroxyl

groups. The part structure (XIII) of Fuerstiaquinone could tautomerise to a dihydroxy naphthalene if C_{10} supports a hydrogen atom and to avoid this it is proposed that the carbon atom (10) does not bear any hydrogen atom. Therefore the structure becomes:

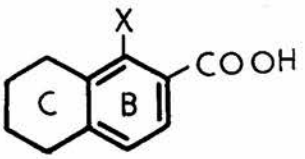


XIV

During the aromatisation of the ring (B), one of the substituents at C_{10} migrates to C_5 with the consequent opening of the labile ring (A) which, on being oxidised, introduces a carboxyl group at the position 4.

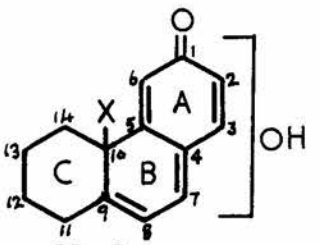
Kuhn-Roth analysis of the compound, $C_{14}H_{18}O_2$, showed that it contained one C-methyl group. The presence of an isolated double bond in the side-chain of the aromatic ring seems unlikely since the acid is an oxidation product. Moreover, the infra-red spectrum of the compound did not show any strong absorption in the regions 995-985 cm.$^{-1}$, 970-960 cm.$^{-1}$, 915-905 cm.$^{-1}$, 895-885 cm.$^{-1}$ and 728-675 cm.$^{-1}$ arising from the = CH or = CH_2 out-of-plane deformation vibrations. It was, therefore, assumed that the compound, $C_{14}H_{18}O_2$ possesses a bicyclic structure (XV) to

satisfy the analysis if there is no double bond. This led to the introduction of another ring (C) in Fuerstiaquinone as shown in the part structure (XVI).



(X = alkyl group)

XV



(X = same alkyl group as in XV)

XVI

It has been mentioned that in order to avoid tautomeric rearrangement, the carbon atom (9) terminating the chromophoric system cannot be linked to a carbon atom which bears hydrogen. Therefore, in the structure (XVI), C_{11} cannot contain hydrogen. Disubstitution would need at least two carbon atoms, leaving only one available for the angular group X. If the ring (C) is five-membered then another carbon atom is available which gives rise to the following cases:

(i) substitution on the ring (B)

(ii) substitution on the ring (C)

(iii) ethyl group as one of the substituents at C_{11}

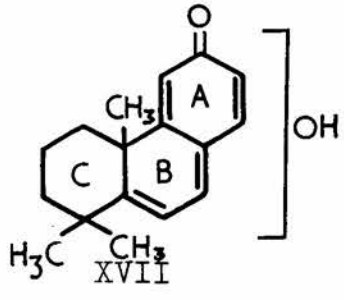
(iv) angular ethyl group

The last case seems very unlikely. If (ii) or (iii) is the case then an asymmetric centre will exist in the compound

$C_{14}H_{18}O_2$ and it will therefore be optically active. But the compound, on the contrary, did not show any optical rotation (.055 M solution was used). If the substitution is on the ring (B), then the bathochromic shift of the ultraviolet maxima (compared to benzoic acid) would be expected to be greater than 10 mμ. Further support to the idea that the two carbon atoms in ring (B) are unsubstituted, was obtained from the infra-red spectrum of $C_{14}H_{18}O_2$.

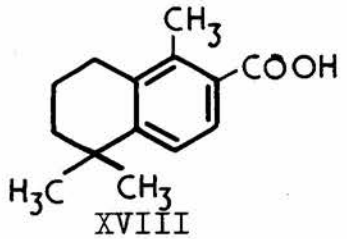
According to Bellamy,[50] two adjacent hydrogen atoms on an aromatic ring cause a strong absorption in the range 860-800 cm.$^{-1}$ due to out-of-plane CH deformation vibration (For example, 1:2:3:4-tetramethyl benzene[51] exhibits a strong band at 804 cm.$^{-1}$). In addition to that weak but sharp absorption bands also occur in the ranges 1225-1175 cm.$^{-1}$, 1175-1125 cm.$^{-1}$ and 1125-1090 cm.$^{-1}$, together with two additional bands in the range 1070-1000 cm.$^{-1}$. In similar fashion the spectrum of the acid, $C_{14}H_{18}O_2$ in nujol showed a strong band at 842 cm.$^{-1}$ and sharp, medium bands at 1176, 1143, 1124, 1074 and 1033 cm.$^{-1}$.

The above facts suggested that the ring (C) is not five-membered, and is therefore six-membered with two methyl substituents at C_{11} and one at C_{10}. In consequence Fuerstiaquinone can be written as:



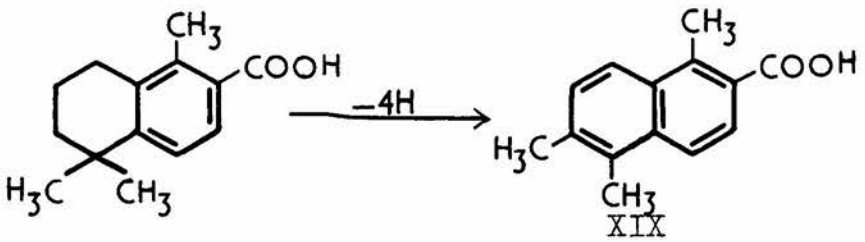
XVII

This leads to the structure (XVIII) for the oxidation product, $C_{14}H_{18}O_2$.



XVIII

Braude, Brook and Linstead[52] have found that tetralin and similar compounds undergo ready dehydroaromatisation on refluxing with chloranil (2:3:5:6-tetrachloro-1:4-benzoquinone) in xylene solution. For example, tetralin was found to be converted into naphthalene in quantitative yield (boiling xylene solution, 20 hr.) It was considered that the compound $C_{14}H_{18}O_2$, should undergo dehydrogenation under similar condition to yield the naphthalene derivative (XIX).



XIX

The methyl ester of $C_{14}H_{18}O_2$ and chloranil in xylene (sulphur free) were refluxed for twenty hours in nitrogen. The reaction

mixture, on chromatographic purification, yielded colourless plates which were found to be identical with the starting material on examination by the X-ray powder photographic method. The dehydrogenation did not take place because the condition was presumably too mild for such a compound (containing gem-dimethyl group).

It should be mentioned here that the main product of zinc dust distillation (i.e. the colourless oil which partly crystallised, see page 45) was also subjected to the same re-:action. On heating with chloranil for forty hours under reflux in an atmosphere of nitrogen, the product was obtained as a wax-like solid (m.p. 50-60°). It seemed that the dehydro-:genation had proceeded to some extent but the ultra-violet spectrum showed only benzenoid absorption ($\lambda^{C_6H_{12}}_{max.}$ 224 mμ, $E^{1\%}_{1\ cm.}$ 90; Fig.IX) Combustion analysis gave: C, 84.82; H, 13.34; mol.wt. 382.

In structure (XVII), seventeen carbon and twenty hydrogen atoms have been accounted for. The molecular formula of Fuerstia-:quinone ($C_{20}H_{26}O_3$) requires three carbon and six hydrogen atoms more. Therefore, a $-C_3H_7$ residue is evidently present as one or more substituents on the ring A.

The colourless solution, obtained by reducing Fuerstia-:quinone with hydrogen in presence of platinum, was treated with a solution of diazotised sulphanilic acid (made slightly alkaline) whereby a deep wine-red colour developed due to coupling.

This implies that the ring (A) must contain a free position. Another significant test is due to Craven.[53] According to him, quinones having a free position in the quinonoid ring, condense with ethylcyanoacetate in presence of ammonia to produce green colourations. On applying this test to Fuerstiaquinone, a positive result (intense bottle green colour) was obtained.

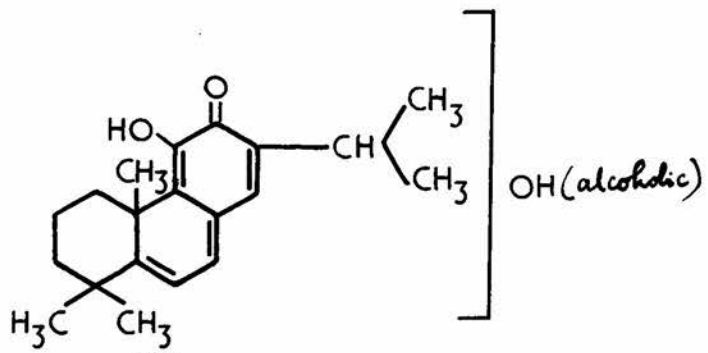
It will be seen in structure (XVII) that only two positions are available for substitution. But for the reasons just mentioned one of these positions must remain free. Therefore, the residue $-C_3H_7$ can only be a propyl or an isopropyl group. Indeed the infra-red spectrum of Fuerstiaquinone gave strong support for the presence of the latter group. Two bands at 1372 cm.$^{-1}$ and 1342 cm.$^{-1}$ (HCB) which arose from the two CH_3 bending vibrations of the isopropyl group were present. This gained confirmation from the $(CH_3)_2$-C skeletal vibration. According to Simpson and Sutherland[54] the isopropyl type skeletal frequencies occur near 1170 and 1145 cm.$^{-1}$. In the series 2-methylpropane to 2-methylnonane they found one frequency to be constant in the range 1170-1167 cm.$^{-1}$ while the second fell steadily from 1170 cm.$^{-1}$ in the first to 1142 cm.$^{-1}$ in the last. The spectra of Fuerstiaquinone showed strong bands at 1168 and 1153 cm.$^{-1}$ in nujol and at 1175 and 1155 cm.$^{-1}$in CCl_4 solution.

It must be mentioned that in addition to these characteristic isopropyl frequencies, the spectrum of Fuerstiaquinone also showed absorptions due to gem. -dimethyl group. The

tertiary butyl group $(CH_3)_3C-$ is known to absorb at 1395 and 1365 cm.$^{-1}$, the intensity of the second band being about twice that of the first.[55] The skeletal vibrations of this group[54] appear near 1250 and 1200 cm.$^{-1}$. The spectrum of Fuerstiaquinone (CCl_4) showed bands at 1390 and 1360 cm.$^{-1}$. The intensity of the second band was almost twice that of the first. In the spectrum, which was determined with nujol smear, a strong and a medium intensity band was also present at 1237 and 1200 cm.$^{-1}$ respectively. There is no indication of a tertiary butyl group in Fuerstiaquinone and it is probable that these absorptions result from the gem-dimethyl group.

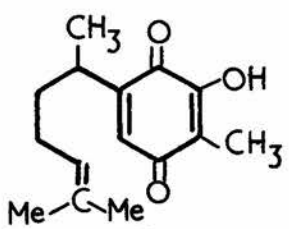
The presence of isopropyl, gem-dimethyl and angular methyl groups suggest that Fuerstiaquinone is a terpenoid compound, and this is in harmony with the formula of the hydrocarbon $C_{20}H_{28}$ (on the basis of one carbonyl group) from which Fuerstiaquinone ($C_{20}H_{26}O_3$) is derived. By analogy with the terpenes, it was recognised that the isopropyl group in Fuerstiaquinone is not substituted at C_6 and therefore must be at C_2 or C_3. The colourless substance, obtained by reducing Fuerstiaquinone with Pt/H_2, did not respond to Gibb's test[56] and therefore suggested that there was no free para-position with respect to the phenolic OH groups in the compound. This is only possible if the isopropyl group is present at C_3; but if that were so mild oxidation of Fuerstiaquinone with alkaline hydrogen peroxide would not produce compound (XVIII) (the product would probably have been

XVIII with CO_2H replaced by $-CO.CH(CH_3)_2$). It is known, however, that the above colour reaction is not always reliable[57] and so the position C_2 was preferred for the isopropyl group. This fixed the position of the hydroxyl group (XVII) at C_6. The structure (diterpenoid) of Fuerstiaquinone can, therefore, be written as:

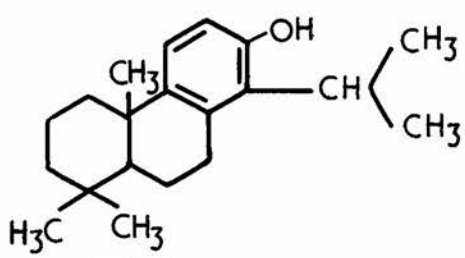


XX

The position of the alcoholic hydroxyl group will be discussed later. Up till now only one terpene quinone (viz. perezone,[58] XXI) is known. It may be noted that the above structure of Fuerstiaquinone closely resembles that of Totarol,[59] a diterpene phenol (XXII).



XXI

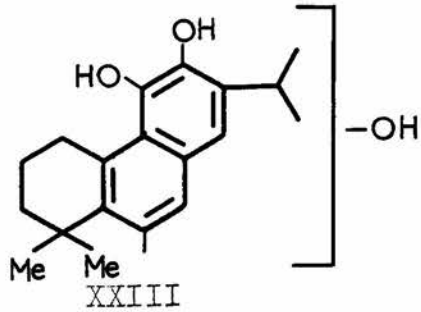


XXII

It is now necessary to consider how far the proposed

structure for Fuerstiaquinone expresses the properties of the compound. A solution of Fuerstiaquinone in ethanol was rendered completely colourless by hydrochloric acid. The rearrangement product was not very stable and the solution gradually turned yellow due to oxidation in air. The oxidation product was found to be different from Fuerstiaquinone. Its visible spectrum showed maximum at 420 mμ and in infra-red it produced only one OH absorption band at 3293 $cm.^{-1}$. When Fuerstiaquinone was decolourised with acid and the ultra-violet spectrum of the colourless solution was quickly determined, a naphthalene derivative was detected (maxima at 341, 308 and 241 mμ with log ε 3.36, 3.69 and 4.82 respectively; Fig.XIV). This compound coupled with diazotised sulphanilic acid producing a deep wine-red colour and also gave a green colour with ferric chloride.

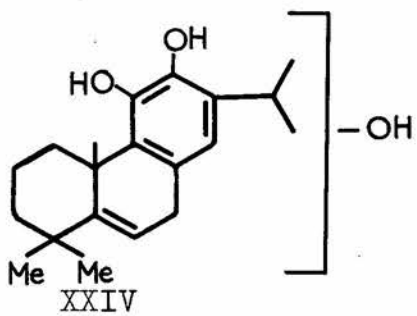
It is realised that during the rearrangement, migration of the angular group takes place, probably at C_8 inducing the formation of the naphthalene derivative from ring (A) and (B). The structure (XXIII) is, therefore, being proposed for the acid rearrangement product.



XXIII

It is probable that this compound oxidises in air producing a β-naphthoquinone derivative (λmax. at 420 mμ) in which one of the carbonyl groups is hydrogen bonded with the alcoholic hydroxyl group (band at 3293 $cm.^{-1}$).

The dihydro-compound, which is obtained by reducing Fuerstiaquinone with hydrogen in presence of Pt catalyst and which forms the triacetate, is to be formulated as (XXIV) in which the additional double bond is not conjugated with the benzenoid ring in accordance with the ultra-violet absorption spectrum (of the triacetate) which is distinct from that of substituted styrene.[60]

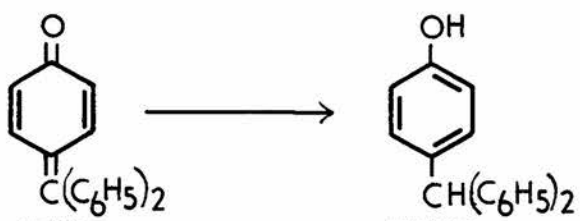


XXIV

This compound coupled with diazotised sulphanilic acid and gave a green ferric reaction indicative of the ortho-dihydroxybenzene system. The ultra-violet spectrum of its triacetyl derivative produced a doublet (at 282 and 292 mμ with log ε 2.7 and 2.8 respectively; Fig.III) similar to that of catechol derivatives.[40] The aromatic ring was also detected by the infra-red spectrum of the reductive triacetate. With the high dispersion of a lithium fluoride prism, two bands at 3066 and

3023 cm.$^{-1}$ were detected. The absorption due to the isolated double bond* was present as a band at 1638 cm.$^{-1}$. The optical activity of the reductive triacetate arose from the asymmetric centre at C_{10}.

On reduction, diphenyl p- benzoquinone methide (XXV) produce p-hydroxytriphenyl methane (XXVI) which is a quite stable compound. It does not change back to the original methylene quinone upon aerial oxidation.[61] The re-oxidation of (XXIV) to Fuerstiaquinone rather than to the corresponding o-quinone can probably be attributed to the stability gained by structure (XVII) by hydrogen bonding between the carbonyl and the hydroxyl group as well as from the incorporation of the additional double bond into the conjugated system.



XXV XXVI

The aromatisation of the ring B (XVII) to produce the peroxide oxidation compound (XV) is not possible merely by the migration of the angular group to C_5. A hydroxyl group must be involved in this process. It was recognised that there are two possibilities for the position occupied by the hydroxyl group, i.e., an angular hydroxymethyl group ($-CH_2OH$) or a hydroxyl group

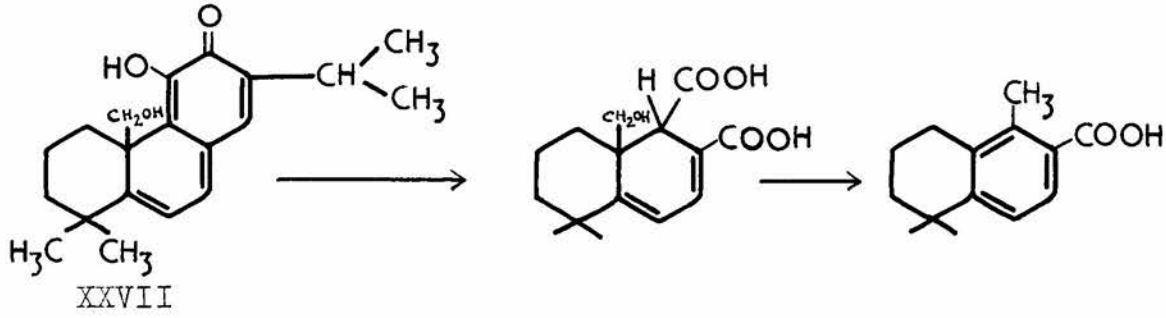
* The reductive triacetate gave positive test for unsaturation with tetranitromethane.

at C_{14}. Both explain satisfactorily the formation of (XV).

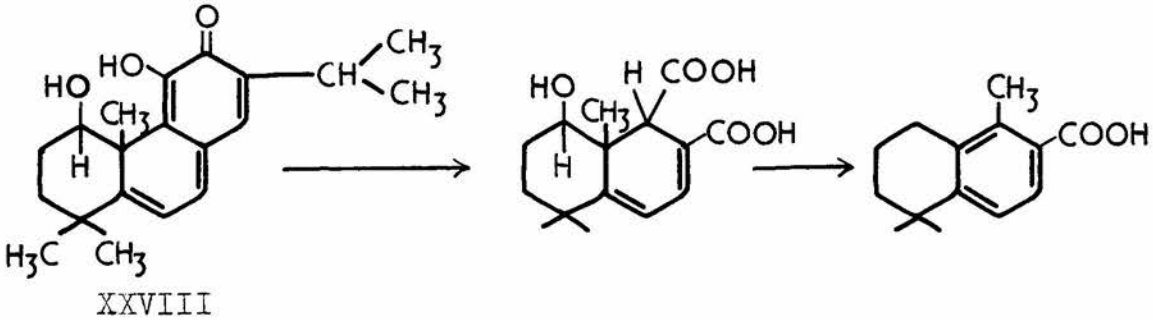
Mc Elvain et al.[62] have made a systematic study on the Kuhn-Roth C-methyl determination and have recorded a large number of data for compounds containing methyl groups in various forms. They have found that the gem-dimethyl of an isopropyl group gives approximately one C-methyl value (isobutyric acid 0.91, isovaleric acid 0.87 etc.). In contrast to this, the gem-dimethyl groups present in dibasic acids have been found remarkably stable to oxidation and for this reason they do not give significant C-methyl values (dimethyl malonic acid 0.10, α,α-dimethyl succinic acid .08 and β,β-dimethyl glutaric acid .01). The similar values observed for some compounds containing gem-dimethyl group in a cyclic system (1,1-dimethyl cyclohexane-3:5-dione-isophorone, β-caryophyllene etc.), have been proved to be due to the oxidation of the substances primarily to dibasic acids of the type just mentioned. It follows that compounds which contain gem-dimethyl in a cyclic system and which are at the same time resistant to oxidation to dibasic acids, would be expected to give almost normal C-methyl values. Mc Elvain et al. have also found that an angular methyl group gives approximately one-half of the theoretical C-methyl value (trans-1-methylcyclopentane-1,2-dicarboxylic acid 0.58, cis-9- methyldecalone-1-semicarbazone 0.56, trans-9-methyldecalone-1-semicarbazone 0.43 etc.).

The Kuhn-Roth analysis of Fuerstiaquinone gave a C-methyl

value of 0.94. Taking the isopropyl group into account, it seems that the presence of an angular methyl group in Fuerstiaquinone would have given a higher value. Therefore the angular group is probably present as hydroxymethyl (XXVII). It must be pointed out that although the structure (XXVIII), containing a hydroxyl group at C_{14} and an angular methyl group, is not consistent with the C-methyl analysis, it can be favoured on the biogenetic ground. The formation of (XV) by peroxide oxidation of (XXVII) and (XXVIII) probably takes place through an intermediate dicarboxylic acid, which undergoes intramolecular loss of water and migration of the angular group with decarboxylation.



XXVII



XXVIII

A methyl group attached to an aromatic ring is known to give only 12% of the theoretical C-methyl value (m-xylene 0.24)[63] The Kuhn-Roth analysis of (XV) gave a C-methyl value of 1.13. This high value is presumably due to the fact that (XV) is not easily oxidised to a dicarboxylic acid containing gem-dimethyl group (and hence a value normal to a gem-dimethyl of an iso-propyl group is obtained; vide supra). In Fuerstiaquinone, this type of oxidation will be expected to go very readily.

It was previously mentioned that, in addition to the acid $C_{14}H_{18}O_2$, two other acids were isolated from the peroxide oxidation product by chromatography on a cellulose column. But none of these could be crystallised. When the oxidation product was methylated with diazomethane and the mixture was first subjected to distillation and sublimation under reduced pressure and then to chromatography on alumina, four esters could be isolated. These are listed below:

(A) colourless fragrant smelling liquid, b.p. ca.80°/11 mm, n_D^{20} 1.4224.

(B) colourless crystalline plates, m.p. 81°.

(C) very pale yellow glass.

(D) white amorphous solid.

Infra-red spectrum of the volatile liquid (A) showed a strong band at 1716 cm.$^{-1}$ (ester carbonyl) and a very strong and broad band extending from 3750 to 3060 cm.$^{-1}$ with maximum at

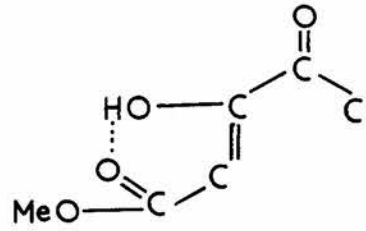
3380 cm.$^{-1}$. The latter suggested the presence of an alcoholic hydroxyl group which, by virtue of polymerisation, caused the broadness of the absorption band. This view gained confirmation from other strong bands in the low frequency region, 1450-1350 and 1150-1050 cm.$^{-1}$.[64] Combustion analysis of the substance gave C, 35.36 and H.8.37%. The liquid is therefore considered to be the ester of a hydroxy acid which, presumably, contains four to eight carbon atoms. This ester was obtained in very small amount and so further investigation could not be made. However, it is apparent that this fragment can originate either from the ring (C) of Fuerstiaquinone or from the iso-propyl side-chain. If latter is the case, then probably the side chain is - CHOH $(CH_3)_2$ (cf. eudesmol and carotol[65]). This would imply that the hydroxyl group involved in the aromatisation process (which leads to the formation of $C_{14}H_{18}O_2$) must have come from hydrogen peroxide. It is important to note that, a hydroxyl group on the side-chain would make the hydrogen bonding possible with the quinone carbonyl which is formed by oxidation of the acid rearrangement product of Fuerstiaquinone.

The substance, obtained by sublimation under reduced pressure, was found to be the methyl ester of the acid, $C_{14}H_{18}O_2$.

The non-crystalline substances (C) and (D) were obtained in good yield (157 mg. and 230 mg. respectively from the oxidation of 1.35 g. of Fuerstiaquinone). Infra-red spectra showed that both these substances are hydroxy esters. The former showed a

strong band at 1750 cm.$^{-1}$ due to ester carbonyl and a slightly broad, medium band with maximum at 3458 cm.$^{-1}$ due to the bonded OH. Similar bands in the spectrum of the latter appeared at 1730 and 3440 cm.$^{-1}$ (broad). The OH absorption band of (D) was much broader than that of (C) proving that a stronger hydrogen bonding existed in the former. The ultra-violet spectra of these substances show that they do not possess aromatic structure (Fig. XII and XIII). (C) showed two maxima at 278 and 246 mμ ($E^{1\%}_{1\ cm.}$ 49.5 and 75 respectively) and (D) only one at 235-240 mμ ($E^{1\%}_{1\ cm.}$ 117). In addition to these, infra-red spectra afforded other valuable informations. Two strong bands at 1683 and 1649 cm.$^{-1}$ in (C) and 1677 and 1648 cm.$^{-1}$ in (D) were also present. The former bands are due to the $\alpha\beta$-unsaturated ketone groups and the latter probably arises from the chelation of a conjugated carbonyl with the hydroxyl group. This carbonyl might be of the ketone or the ester. For example, ethyl $\alpha\alpha$-dimethylaceto-acetate, which cannot enolise, shows absorptions at 1742 and 1718 cm.$^{-1}$ due to ester and ketone carbonyl groups, but both ethyl α-methyl-:acetoacetate and ethyl acetoacetate show an additional band at 1650 cm.$^{-1}$, which is ascribed to the ester carbonyl group after chelation to the enolic hydroxyl group.[66] It is probable that (C) and (D) possess the following type of system (XXIX) origin-

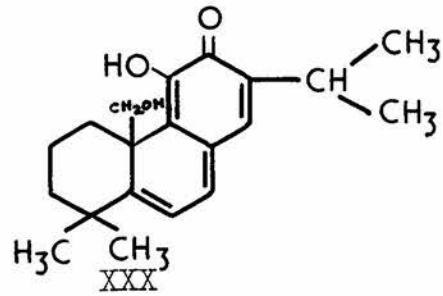
:ating from the ring (A) of Fuerstiaquinone.



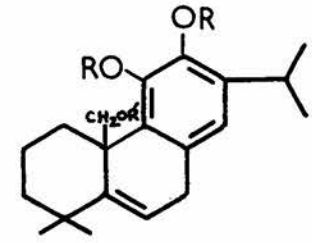
XXIX

None of the compounds (C) and (D) produced any colour with ferric chloride. From an inspection of their infra-red spectra, it seemed that the two compounds were very similar. Any apprec-:iable difference in the spectra existed only in the region 1250-750 cm.$^{-1}$, especially 1000-750 cm.$^{-1}$. The strong bands at 1141 and 883 cm.$^{-1}$ in the spectrum of (D) were absent in that of (C).

Theoretical considerations and experimental findings lead to the conclusion that the alcoholic hydroxyl group in Fuerstiaquinone is most probably present in the form of the angular hydroxymethyl group, but final proof requires further experimental data. With this reservation the structures (XXX), (XXXI) and (XXXII) are advanced for Fuerstiaquinone, its reductive triacetate and reductive dimethyl ether respectively.

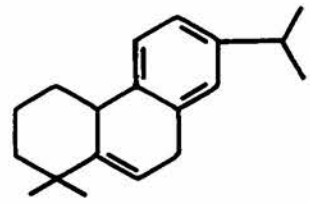


XXX



XXXI (R and R' = Ac)
XXXII (R = Me and R' = H)

From the above structure of Fuerstiaquinone it is now understandable why the zinc dust distillation product was not fully aromatic. As soon as the ring (A) aromatised, the resulting substance, being very volatile, was removed from the contact with zinc dust and so complete dehydrogenation could not take place. It is probable that the colourless oil (obtained as the main product of the distillation) possesses the structure (XXXIII).

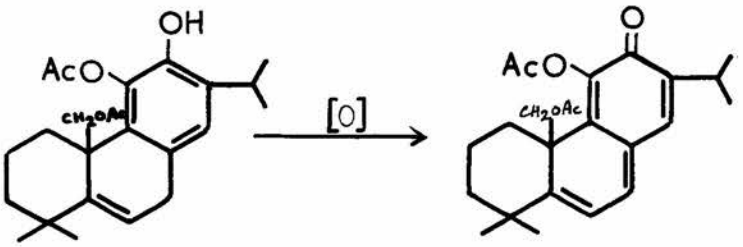


XXXIII

It can now be suggested that complete dehydrogenation will only be achieved by heating Fuerstiaquinone with zinc dust in a sealed tube. Selenium, which is normally employed for dehydrogenating the terpenes, will probably give much better results.

The reductive diacetate of Fuerstiaquinone, obtained by the action of zinc dust, acetic anhydride and triethylamine probably has the structure (XXXIV). The colour change (from white to orange) that takes place on keeping the substance is

then due to the slow oxidation as shown below:



XXXIV

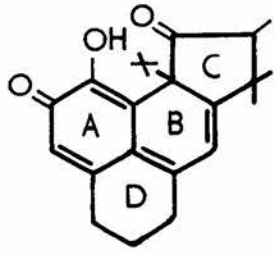
It is, however, difficult to understand why one hydroxyl group is preferentially acetylated. In agreement with this structure, the infra-red spectrum of the diacetate showed two strong bands at 1778 and 1750 cm.$^{-1}$ (due to vinyl and normal ester carbonyl respectively) and a medium band at 3500 cm.$^{-1}$.

Lastly it should be mentioned that the infra-red spectrum of Fuerstiaquinone (in nujol) showed a medium intense band at 1563 cm.$^{-1}$ and a very weak one at 1635 cm.$^{-1}$. These are due to the C=C stretching vibration of the conjugated double bond system. Jones *et al.*[67] have recorded similar observation in the case of sterols containing conjugated dienes. They found all such materials to produce two maxima due to C=C stretching vibration. For example, a $\Delta^{3:5}$-sterol absorbed at 1618 and 1578 cm.$^{-1}$. The infra-red spectrum of Fuerstiaquinone also showed strong bands at 2984, 2956 and 2878 cm.$^{-1}$, and a medium band at 2861 cm.$^{-1}$ when determined by using lithium fluoride prism. The bands at 2984 and 2878 cm.$^{-1}$ were due to the CH stretching vibrations of the CH_3 group and those at 2956 and 2861 cm.$^{-1}$ arose from the

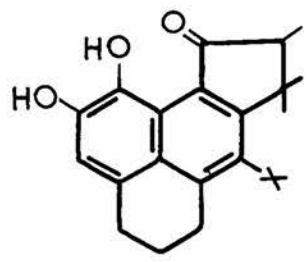
similar vibration of the CH_2 group.

An interesting property of Fuerstiaquinone requires mention. The ultra-violet and visible spectra of Fuerstiaquinone were determined with a solution of the substance in spectroscopic ethanol when a maximum at 435 mμ and an inflexion at 260 mμ (with log ε 4.1 and 3.4 respectively) were obtained. This solution was preserved in glass stoppered bottle and after one year and nine months the spectrum was again determined. This time an entirely different type of absorption occured. Two maxima were found at 330-335 mμ and 250 mμ (log ε 3.7 and 4.04 respectively) and the shape of the curve (Fig. XV) was very similar to that of α-naphthaquinone (p.17; $\lambda_{max.}$ 334, 256 and 246 mμ with log ε 3.44, 4.13 and 4.28 respectively). An understanding of this spontaneous rearrangement in neutral medium might provide valuable information about the chromophore proposed in this text for Fuerstiaquinone.

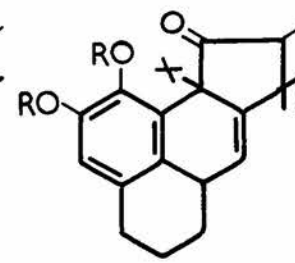
It is remarkable that in a paper published very recently Johnson and Grant[68] have proposed the same chromophore for pristimerin as is proposed here for Fuerstiaquinone. According to these workers pristimerin (XXXV), its acid rearrangement product (XXXVI), dihydrocompound (pristimerol, XXXVII), reductive diacetate (XXXVIII) and the Thiele acetylation product (XXXIX) possess the structures shown below:



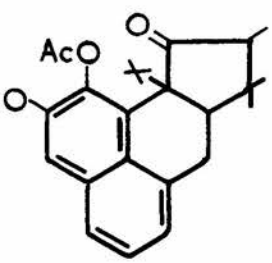
XXXV



XXXVI

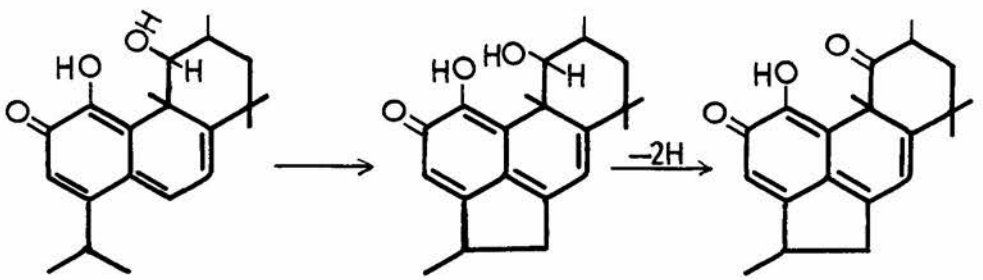


XXXVII (R = H)
XXXVIII (R = Ac)



XXXIX
(X = Me or OMe)

The structure (XXXV) of pristimerin is strikingly similar to that of Fuerstiaquinone and it is of particular interest to note that if (C) and (D) are actually present as a six-membered and a five-membered ring respectively in pristimerin, rather than in the opposite way as represented in the above structure, then Fuerstiaquinone (as shown in XXVIII) can be regarded as a dihydro pristimerin type compound (the C=O group in ring C being reduced to -CHOH-). It is possible for the iso-propyl group to be present at C_3 (cf. Totarol)[59] and to cyclise with the C_7 to produce a five-membered ring as shown below:



The only evidence presented by Johnson for the five-membered nature of the ring (C) is the C=O stretching frequency which appears at 1729 $cm.^{-1}$. But it can be pointed out that this value is more consistent with a six-membered rather than a five-membered ring.

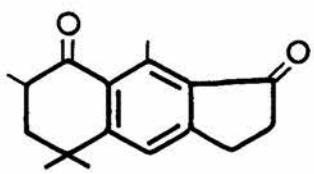
It is to be expected that the carbonyl frequencies of strained five-membered rings will be higher than those of six-membered rings. Jones et al.[26b] have examined a very large number

of steroids containing carbonyl groups in six-membered and five-membered rings, and they have found that the carbonyl frequencies of all the former remain within the range 1720-1706 cm.$^{-1}$ unless conjugation effects occur. In twenty two compounds which contain carbonyl groups (non-conjugated) in five-membered ring, they found this range to be 1749-1 745 cm.$^{-1}$. On this basis Jones has been able to differentiate carbonyl groups in five- and six-membered rings. It seems probable that the ring (C) in pristimerin (XXXV) is six-membered.

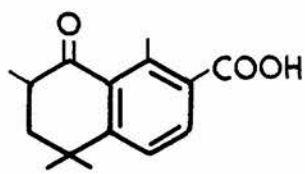
The ultra-violet spectrum of the acid rearrangement product of pristimerin was similar to that of naphthalene derivatives (λmax. at 333, 317, 294 and 238 mμ with log ϵ 3.45, 3.46, 3.84, and 4.78 respectively). For comparison, the similar product of Fuerstiaquinone showed maxima at 341, 308, and 241 mμ with log ϵ 3.36, 3.69, and 4.82 respectively. The carbonyl frequency of the acid rearrangement product of pristimerin (XXXVI) was found to appear at 1684 cm.$^{-1}$. This lowering was due to the conjugation produced upon rearrangement of the molecule. But in the Thiele acetylation product (XXXIX) the normal carbonyl frequency was present (at 1729 cm.$^{-1}$). These facts together with the naphthalenoid nature of the Thiele acetylation product (as revealed by its ultra-violet spectrum) led Johnson to propose the peri-naphthalene ring (D).[68] It should be pointed out that this is not a rigid proof and so the presence of the ring (D) can be related with some doubt. Moreover, the formation of picene upon

zinc dust distillation of pristimerin cannot be reconciled with this pyrene type structure (XXXV) as presented by Johnson.

However, the point to be derived at is that, the ring (C) in pristimerin is six-membered and the ring (D) does not exist; but if it does, it is probably five-membered. From one aspect, of course, it seems that C_7 in pristimerin is substituted. This is the comparatively greater stability of the dihydrocompound of pristimerin (XXXVII) than that of Fuerstiaquinone (XXIV). Substitution at C_7 probably creates steric hindrance and therefore makes this centre less vulnerable to oxidative attack. However, it is suggested that the product from the alkaline peroxide oxidation reaction of pristimerin would make this point clear. If the ring (D) exists, a compound of the type (XL) will be produced. Otherwise, one would expect a compound similar to the oxidation product ($C_{14}H_{18}O_2$) of Fuerstiaquinone as shown in (XLI).



XL



XLI

Lastly, it is to be mentioned that the oxidation of Fuerstiaquinone with pot. permanganate, nitric acid etc. as well as the ozonolysis reaction were carried out, but it was not possible to investigate

the products from these reactions to an extent that could disclose some additional facts consistent with the proposed structure of Fuerstiaquinone. However, it seems desirable to describe the general findings here.

Ozonolysis of Fuerstiaquinone affords a water-soluble , colourless, crystalline acid, which decomposes at about 110° with the liberation of CO_2. Unlike the barium salt, the silver salt of the acid is insoluble in water. Its behaviour in paper chromatography (R_f 0 in the solvent, n-BuOH saturated with 1.5N NH_4OH) suggests that it is a di- or tri-carboxylic acid. Infra-red spectrum shows that a hydroxyl group (3500 $cm.^{-1}$) is present as well in the molecule.

In addition to this acid, the ozonolysis reaction produces three other substances (R_f 0.94, 0.61 and 0.21 in n-BuOH saturated with 1.5N NH_4OH). These do not respond to fluorescein test. The mixture forms a semicarbazone and therefore at least one of the three components contains a ketonic group.

No formaldehyde is produced in the ozonolysis reaction.

Oxidation of Fuerstiaquinone with hydrogen peroxide in presence of ferrous sulphate catalyst, proceeds very slowly with the discharge of red colour. The product is difficult to purify.

Permanganate oxidation gives a very viscous yellow oil having smell like cyclohexanol. It proves to be a mixture of four substances (R_f 0.86, 0.63, 0.57 and 0.17 in n-BuOH saturated with 1.5 N NH_4OH). Comparatively small amount of a white solid

crystallises out from a solution of the mixture in methanol. The mixture does not respond to Fluorescein test.

Oxidation of Fuerstiaquinone with nitric acid gave a viscous oil which was found to be a mixture of at least five substances (R_f 0.76, 0.66, 0.53, 0.28 and 0-0.19 in n-BuOH saturated with 1.5 N NH_4OH). The product gave positive fluorescein test but nothing could be sublimed from it under high vacuum. A colourless crystalline acid, m.p. 205° (decomp.) was obtained from the oxidation mixture. This was found to lose water at 75° (when the crystals turned milky-white most probably due to the formation of the anhydride) and to decompose at 170°. It also responded to the Fluorescein test. Analysis of the acid gave C, 20.89, H,3.70; N, 6.34. This could only be explained by a structure based on $C_4H_5NO_9$, possibly $(HOOC)_3C\text{-}NO_2.H_2O$.

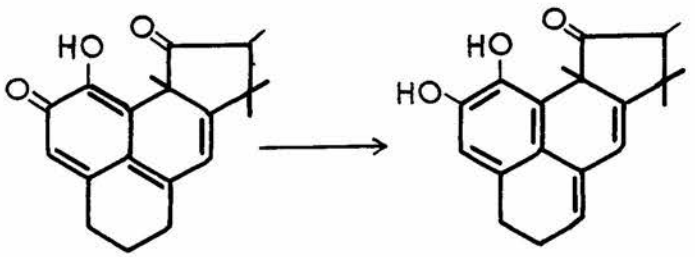
Through a private communication with Dr. R.H. Thomson (Aberdeen), it is learnt that he as well as Dr. R.G. Cooke (Melbourne) are of opinion that the following features of Johnson's structure for pristimerin are unsatisfactory.

(1) the 'inert carbonyl group'

(2) there seems to be no reasonable way of including the hydrolysable methoxyl group in the chromophore.

(3) the saturated ring of the perinaphthane skeleton should lose a proton and allow

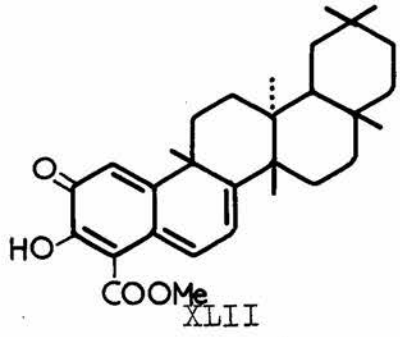
reversion to an aromatic ring, either spontaneously or very readily in the presence of a trace of acid or alkali.



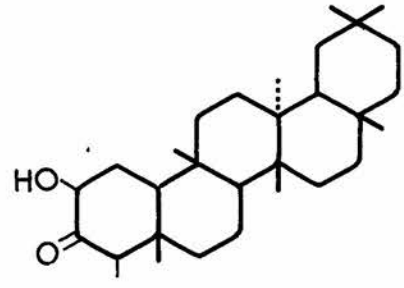
(4) the acid rearrangement product cannot have the naphthalene-1:2-diol system suggested because it could not couple to form an azo dye and would not be stable in alkali (cf. Fuerstiaquinone)

According to Cooke and Thomson, the 'inert carbonyl group' and the methoxyl group are combined together in the form of an ester group, a fact which accommodates all the infra-red data. The drop in frequency in pristimerol, and in the acid rearrange-:ment product is to be attributed to hydrogen bonding in a sali-:cylic type of ester. The pentacyclic structure (XLII) of pristimerin proposed by them assumes a relationship to the triterpenes (dehydrogenation to an alkylpicene) of the friede-:lane group (XLIII) which have been found in the same plant

family.[69]

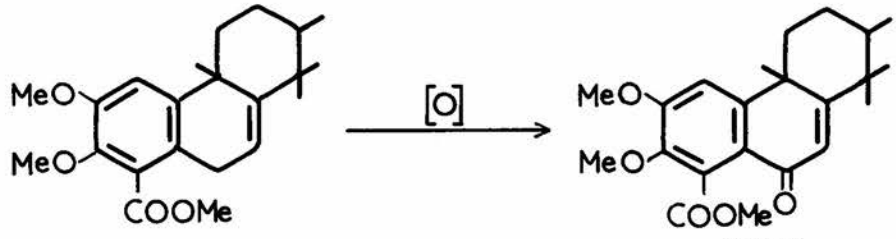


XLII



XLIII

The oxidation product from pristimerol dimethyl ether has been suggested to be (XLIV) (Johnson's interpretation does not accord with the molecular formula he reports).



XLIV

The author entirely agrees with these ideas of Cooke and Thomson and believes that such a structure (XLII) of pristimerin provides a strong support for the proposed structure of Fuerstia-
:quinone.

EXPERIMENTAL

The following notes apply to this as well as to the experimental section of Part II.

Unless otherwise stated, evaporations were carried out under reduced pressure, and the ligroin had b.p. 40-60°.

Melting points were determined by means of a Kofler micro-melting point apparatus.

Analyses were performed by Drs. Weiler and Strauss, Oxford.

Alumina used for chromatography was supplied by Peter Spence & Sons Ltd., Widnes (Activated Alumina, Type 'H', 100/200S mesh).

Hanovia lamp was used for the observations in ultra-violet light.

Paper partition chromatography was effected on Whatman No.1 filter paper.

Ultra-violet spectra were determined with a Unicam SP 500 spectrophotometer. Unless otherwise stated, ethanolic solutions were used. Absolute ethanol was purified by refluxing with sodium (10 g. to a winchester) for three hours followed by distillation.

Infra-red data were obtained with a Hilger H 800 double beam spectrometer.

Fuerstiaquinone:- For isolation, see page 10. Fuerstiaquinone (first obtained from ligroin solution) was recrystallised thrice from iso-octane (2:2:4-trimethylpentane) and was then dried (2 hours) over P_2O_5 in vacuo at 56°. The dried substance, m.p. 108-109°, gave on analysis: C, 76.34, 76.07; H,7.95, 8.07; C-CH_3, 4.50% (0.94 group); active H, 0.48% (1.5 group); N and OCH_3 absent. $C_{20}H_{26}O_3$ requires C, 76.40; H, 8.34; 1 C-CH_3, 4.77%; 1 active H, 0.32%

Ultra-violet spectrum: $\lambda_{infl.}$ 260 mμ (log ε 3.4), $\lambda_{max.}$ 435 mμ (log ε 4.1); Fig.I.

Infra-red spectra:

(a) CCl_4 - 3550 m, 3350 m, 3000 s, 2960 s, 2896 m, 2000 w, 1860 w, 1450 s, 1390 s, 1360 s, 1300 s, 1175 s, 1155 s, 1115 m, 1075 s, 1062 s, and 896 s cm.$^{-1}$.

(b) CCl_4 (LiF prism) - 3480, 3306, 2984 s, 2956 s, 2878 s, and 2861 m cm.$^{-1}$.

(c) HCB - 3530 m, 3350 m, 2980 s, 2945 s, 2885 m, 1463 s, 1454 s, 1400 m, 1372 s, 1342 s, 1287 s, 1271 s, and 1233 m cm.$^{-1}$.

(d) Nujol - 3530 m, 3310 m, 1635 w, 1590 s, 1563 m, 1509 s, 1334 s, 1284 s, 1267 s, 1237 s, 1200 m, 1168 s, 1153 s, 1136 m, 1088 m, 1075 m, 1056 m, 1025 m, 1005 m, 995 m, 945 m, 912 w, 900 m, 890 m, 852 m, 815 m, 805 m, 762 w, and 720 m cm.$^{-1}$.

Molecular weight determination (by Dr. C.A. Beevers)

The crystals consisted of dark red blocks. Oscillation and

Weissenberg photographs were taken about an axis of the monoclinic cell and the reciprocal lattice vectors measured to an accuracy of about 1%. The cell volume was found as 682 $\mathring{A}^3$ and the density (by floatation) was 1.24 (±1% say). Hence the unit cell contents turned out to have a molecular weight of 5090. Thus possible values of M are:

No. of Mols. in cell	M
2	2545
4	1272
8	636
16	318

The space-group is probably $P2_1$.

Colour reactions

(a) Dimroth's test - Boric acid (1 g.) was mixed with acetic anhydride (4.9 g.) and the mixture was warmed on the water bath at 80°. A vigorous reaction started whereby the liquid began to boil and boric acid went into solution. The solution, on cooling, turned into a solid mass (boroacetic anhydride) which was dissolved in some more acetic anhydride.

On adding a few drops of the above reagent[70] to a dilute solution (yellow, 2 ml.) of Fuerstiaquinone in acetic anhydride, a red colour developed which after sometime (ca. 15 min.) turned into a persistent brown-red colour.

(b) Titanous chloride - A dilute solution of Fuerstia-

:quinone in methanol was treated with aqueous titanous chloride solution when a dark red colour was observed. After sometime the colour changed into brown.

(c) Ferric chloride - An ethanolic solution of Fuerstia-:quinone, on treatment with aqueous ferric chloride solution, produced a dark green colour.

(d) Sodium bisulphite - An ethanolic solution of Fuerstiaquinone was treated with a saturated aqueous solution of sodium bisulphite when thered colour of the quinone completely disappeared. On adding hydrochloric acid to the colourless solution, a transient pale violet colour was seen. Instead of the acid, when sodium carbonate (solid) was added to the colourless solution, a pale violet colour was produced which lasted for about half an hour. After that the solution was colourless again.

(e) Ethylenediamine test[31] - Two drops of ethylenediamine were added to a dilute solution of Fuerstiaquinone in ligroin (3 ml.). No red colour developed except that the solution turned colourless. Under the same condition p-benzoquinone produced a red colour.

(f) Craven test[53] - Ammonia gas was passed through an ethanolic solution of Fuerstiaquinone and the resulting pale brown solution was treated with ethyl cyanoacetate. At first, the mixture turned colourless and then an intense bottle-green colour gradually developed. After sometime (ca. 30 min.) this

colour changed into dirty brown.

This experiment was repeated with celastrol. On passing ammonia gas through an ethanolic solution of the compound, a dark brown solution was obtained which, on treatment with ethyl cyanoacetate, did not produce any green colour but became colour-:less.

(g) Action of alkalies - Dil. NaOH solution (aqueous) was added to a methanolic solution of Fuerstiaquinone. At first, a violet-blue colourwas formed which quickly disappeared producing a colourless solution. This, on careful acidification with dil. HCl, regained the red colour and with excess of acid turned colourless again.

(h) Action of acids - Dil. HCl was added to a methanolic solution of Fuerstiaquinone. On shaking the mixture for a few seconds, the colour was completely discharged. This reaction is not reversible because, on treatment with alkali, the red colour did not reappear.

(i) Bromine - One drop of bromine was added to a solution of Fuerstiaquinone (10 mg.) in carbon tetrachloride (5.ml.). Complete decolourisation took place very rapidly.

Acid Rearrangement

(i) A solution of Fuerstiaquinone (50 mg.) in ethanol (10 ml.) was completely decolourised by adding the minimum amount of dil. hydrochloric acid (too much acid would cause precipitation of Fuerstiaquinone). The colourless solution was treated with

excess of water when a white solid separated out. This was extracted twice with ligroin (2 x 50 ml.) and the combined extract, on being freed from the solvent, afforded a colourless oil. The oil did not crystallise. When cooled in dry ice/acetone, it changed into a white solid mass which, on attaining the room temperature, again turned into the original state.

The oil was found to undergo slow oxidation in air; at first its colour changed to pale yellow and that gradually became more and more intense. When the oil was brownish yellow in colour, it was dissolved in ethanol and the solution was left exposed to air overnight, whereby a brownish red solution was obtained. A portion of this solution was treated with dil. HCl but the colour did not disappear, proving that the oxidation pro-:duct was not identical with Fuerstiaquinone. The ultra-violet spectrum of the oxidised substance in ethanol showed maximum at 420 mμ. Infra-red spectrum of the same substance in CCl_4 showed bands at 3293 m, 2971 s, 2925 s, 2877 m, 1762 s, 1728 s, 1720 s, 1666 m, 1467 s, 1452 s, 1408 s, 1383 s, 1371 s, 1346 s, 1317 s, 1117 s, 1071 s, and 1063 s $cm.^{-1}$.

(ii) Few drops of hydrochloric acid were added to a solution of Fuerstiaquinone (2.5 mg.) in spectroscopic ethanol (ca. 5 ml.). When the colour was completely discharged the volume of the solution was made upto 25 ml. and the ultra-violet spectrum was quickly determined: $\lambda_{max.}$ 341, 308, and 241 mμ ($\log \epsilon$ 3.36, 3.69, and 4.82 respectively); λ min. 334 and 273 mμ ($\log \epsilon$

3.32 and 3.33 respectively); Fig. XIV.

The colourless solution of the acid rearrangement product and a solution of diazotised sulphanilic acid were made slightly alkaline and then mixed. A deep wine-red colour developed due to coupling. The rearrangement product also gave a green colour with ferric chloride. The solution of the rearrangement product gradually turned yellow due to oxidation in air.

Condensation with o-phenylene diamine

(i)* A 2 Molar Solution of o-phenylene diamine in hot ethanol (2 ml.) was added to a solution of Fuerstiaquinone (150 mg.) in glacial acetic acid (3 ml.) and the mixture was allowed to stand overnight. The resulting dark red solution was poured into a large volume of water when a wine-red flocculent precipi-:tate was obtained. This was filtered, washed several times with water and then dried. The dried powder (reddish black) did not possess a characteristic melting point and was therefore impure. It was very soluble in ethanol and benzene giving dark solutions. It could not be crystallised.

(ii) A saturated solution of o-phenylene diamine in ethanol (3 ml.) was added to a solution of Fuerstiaquinone (50 mg.) in glacial acetic acid (0.5 ml.) and the mixture was allowed to stand** overnight. The dark red solution was then poured into

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* cf. Tanshinone.[71]

** If the mixture is heated then, on pouring it into water, a chocolate-grey coloured precipitate is obtained.

water when a yellow solid separated out. This was extracted with ether and the ethereal solution (yellow) was first washed with sodium bicarbonate solution, then with water and finally dried with anhydrous sodium sulphate. Removal of ether gave an oil which did not crystallise. On attempting crystallisation of the oil from aqueous ethanol, only a dark grey coloured amor-:phous powder was obtained.

In another experiment, the yellow solid was filtered, washed with water and dried. A yellowish grey powder was thus obtained. It was very soluble in benzene and ethanol (in which it gave dark solutions) and partly soluble in ligroin (yellow solution). It could not be crystallised from ligroin or ethanol-:ligroin.

(iii) A saturated solution of o-phenylene diamine in ethanol (2 ml.) was added to a solution of Fuerstiaquinone (ca. 40 mg.) in ethanol (1 ml.) and the mixture was warmed on the water bath (60°) for half an hour. It was then poured into water and the separated solid was filtered, washed thoroughly with water and dried. A chocolate coloured amorphous powder, m.p. 46°, was thus obtained. Analysis of the substance proved that it was not the desired derivative (Found: N, 1.22%. $C_{26}H_{30}ON_2$ requires N, 7.25%).

Acetylation with acetic anhydride and pyridine

(i) Acetic anhydride (2 ml.) was added to a solution of

Fuerstiaquinone (40 mg.) in pyridine (distilled, 2 ml.). The mixture, on standing at the room temperature for two days, changed its colour from red to yellowish brown. After standing for three more days, it was poured into water and the mixture was stirred well. A yellow solid separated which was filtered, washed several times with water, and then dried in vacuum desiccator over silica gel. The brownish yellow amorphous powder thus obtained melted at 75-85°. The crude product could not be crys-:tallised as it was very soluble in all common organic solvents except ligroin in which it was completely insoluble. Crystallis-:ation attempted from benzene-ligroin and ethanol-ligroin was unsuccessful.

(ii) Pyridine (1 ml.) was mixed with a solution of Fuerstiaquinone (90 mg.) in acetic anhydride (1 ml.) and the mixture, after standing for about a week, was poured into water. The separated yellow solid was extracted with ether and the ethereal solution was washed with distilled water and then with a dilute solution of cadmium chloride (to remove the last trace of pyridine). Removal of ether from the dried (Na_2SO_4) solution yielded a yellow oil which was very soluble in all common organic solvents except ligroin. The oil could not be crystallised from aqueous methanol, benzene-ligroin ethanol-ligroin. On keeping exposed to air, the oil was found to change its colour to dark brown.

(iii) A mixture of Fuerstiaquinone (50 mg.) acetic

anhydride (3 ml.) and pyridine (1 ml.) was boiled under reflux for about two hours. The colour changed to dark brown. The solution was treated as in (ii) but no crystalline solid was obtained except a crude dark coloured amorphous powder.

Thiele acetylation

(i) One drop of conc. H_2SO_4 was added to an ice-cooled solution of Fuerstiaquinone (250 mg.) in acetic anhydride (3 ml.). The colour of the solution first changed to violet-blue which quickly disappeared giving a colourless solution. This was poured into water (25 ml.) and the mixture was stirred. The separated solid was filtered under suction, washed thoroughly with water and then dried. A buff coloured powder, m.p. 65-69° was obtained. It was very soluble in benzene, methanol, ethanol, ether, acetone, chloroform etc. and so could not be crystallised. On treatment with ligroin the substance left a small amount of insoluble residue which crystallised from aqueous ethanol in the form of long colourless needles. The extremely small amount of the crystalline material (only few needles) did not permit investigation.

(ii) One drop of conc. H_2SO_4 was added to a cooled solution of Fuerstiaquinone (130 mg.) in acetic anhydride (5 ml.) and the colourless solution thus obtained was poured into water (100 ml.) with stirring. The resulting mixture was shaken with ligroin in a separating funnel and the ligroin layer was removed. The remaining aqueous phase was extracted with ether and the

extract was well washed with an aqueous solution of sodium bi-:carbonate and then with water. The ethereal solution was dried (Na_2SO_4) and the ether was removed. Very small amount of a colourless needle-like crystalline solid was obtained. This was insufficient for investigation.

The ligroin extract yielded a brownish yellow oil which could not be crystallised.

(iii) In another experiment, the acetylation product was first extracted with benzene and then with ether (instead of ligroin and then ether as in (ii)). The ethereal solution was washed ($NaHCO_3$ and water) and dried (Na_2SO_4) and the ether removed. In this case also, beautiful colourless needles were obtained in very poor yield.

Acetylation with Ketene[24]

Ketene (generated by cracking acetic anhydride at 400°C) was passed through a solution of freshly prepared Fuerstiaquinone (50 mg.) in ligroin (40 ml.) contained in a Drechsel bottle (100 ml.). Within a few minutes the colour of the solution deepened to dark red. The gas was not passed any more and the solution, which smelled strongly of ketene, was allowed to stand overnight at the room temperature. Next day it was washed with a saturated aq. sodium bi-carbonate solution and then with water. When thesolution was shaken with water, the dark red colour quickly changed into dark brown. The solution was dried (Na_2SO_4) and the solvent was removed. A viscous brown oil was obtained. This

was again dissolved in ligroin and the solution chromatographed on alumina using ligroin as the eluting agent. Two bands were found to separate, i.e., an upper brown and a lower yellow band. The lower band, on elution, gave a pale yellow solution which, on standing overnight, went colourless. Removal of the solvent gave a negligible amount of a colourless oil. The upper brown band was eluted with benzene and the eluate was freed from the solvent whereby a dark brown oil was obtained. This could not be crystall-:ised. Infra-red spectrum of the material showed no absorption due to hydroxyl group.

Methylation with methyl iodide and pot. carbonate -

(i) Fuerstiaquinone (80 mg.), acetone (6 ml., distilled after standing with anhyd. $CaCl_2$), methyl iodide (0.5 ml., distilled after standing with P_2O_5) and potassium carbonate (ca. 200 mg., fused) were refluxed for two hours by heating on a water-bath (ca. 90^0). The condenser was protected by a $CaCl_2$ tube. The colour of the solution first changed to reddish yellow and then dark brown. After two hours refluxing, more methyl iodide (0.5 ml.) was added and the mixture further refluxed for one hour. The cooled mixture was filtered and the filtrate, on being freed from acetone and methyl iodide, left a dark brown oil as the residue. This could not be crystallised. The oil was slightly warmed with ligroin whereby most of it went into solution. (The small amount of the insoluble material was dissolved in chloroform and the resulting dark solution was

chromatographed on alumina, but no purification was effected). The ligroin solution was chromatographed through an alumina column (8 x 1 cm.) and elution was continued with ligroin containing increasing amounts of chloroform. On eluting with 20% chloroform-ligroin, a dark brown band (brownish red in ultra-violet) appeared below the blue-violet band (probably due to Fuerstiaquinone) which remained sticking at the top of the column. The lower band was washed out from the column with 40% chloroform-ligroin. Removal of the solvents from the eluate produced a dark oil which was easily soluble in all common solvents. It could not also be crystallised from aqueous methanol or ethanol. Excess of water was added to a solution in methanol and the precipitated solid was filtered, washed and dried in the desiccator. A dark, slightly red amorphous powder was obtained which melted at 43-50°. (Found: C, 73.06; H, 8.30; OCH_3, 12.26%. $C_{20}H_{24}O(OCH_3)_2$ requires C, 77.01; H, 8.77; OCH_3, 18.1%. $C_{20}H_{25}O_2(OCH_3)$ requires C, 77.30; H, 7.97; OCH_3 9.45%). The infra-red spectrum of the substance did not show any OH-absorption.

(ii) Fuerstiaquinone (50 mg.), methyl iodide (5 ml.) and potassium carbonate (ca. 125 mg., fused) were refluxed for three hours. The colour of the solution changed from red to yellow and then dark brown. After cooling, the solution was filtered and methyl iodide distilled off. The residual oil was dissovled in ligroin and the solution chromatographed on magnesium carbonate (heavy). On eluting with ligroin, a dark brown band separated

which moved very slowly. This band was washed off with 30% benzene-ligroin and the solvents were removed. The residual dark oil could not be crystallised.

Methylation with diazomethane

Diazomethane (ca. 125 mg.) in ether (25 ml.) and Fuerstiaquinone (30 mg.) in ether (10 ml.) were mixed. The colour of the solution immediately turned very dark and no nitrogen was evolved. The mixture was allowed to stand overnight. Next day, after boiling off the excess diazomethane, the solution was filtered and the ether removed. A thick yellowish brown oil was obtained. This was easily soluble in methanol, benzene and ethyl acetate but insoluble in ligroin and iso-octane. The oil was dissolved in benzene and chromatographed on magnesium carbonate (heavy). On eluting with benzene, two bands appeared, i.e., a brown (upper) and a pale blue band. They were washed off with 10% chloroform-benzene but no crystalline material could be obtained from the eluates.

In another experiment it was noticed that, when the mixture of diazomethane and Fuerstiaquinone was allowed to stand for 2-3 days, the dark colour of the solution gradually changed to dark brown, yellowish brown, yellowish dark, greenish dark and finally in about a week's time became a madiera colour.

Reduction with potassium borohydride (cf. pristimerol[16b])

A small amount of potassium borohydride was added to a

solution of Fuerstiaquinone (45 mg.) in ethanol (2 ml.). The mixture quickly became colourless. The excess reagent was decom-:posed with acetic acid (3 drops) and hot water was added dropwise to the boiling alcoholic solution until it became slightly turbid. On cooling, a chocolate brown coloured amorphous solid separated which was collected and dried. It melted within a wide range of temperature. It was very soluble in methanol, ethanol, acetone and dioxane but insoluble in ligroin and iso-octane.

The same observation was recorded when water was used instead of acetic acid. It was found that, on adding water, to the decolourised solution, the reduction product precipitated as a milky white solid but it darkened very quickly (within a minute) due to oxidation in air. In one experiment, the reduction and precipitation was done in an atmosphere of nitrogen and a white solid was obtained. But during filtration and drying, the solid darkened again.

Reduction with hydrogen in presence of platinum -

Fuerstiaquinone (30 mg.) in ethanol (10 ml.) was hydro-:genated over PtO_2 catalyst and the solution, within 10-15 minutes, became completely colourless.* This was poured into water when a yellowish white solid separated which gradually changed into brown and then red, due to rapid oxidation in air. The precipitated solid gave a purple colour on treatment with alkali. This, with

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* If this colourless solution is exposed to air for about 5 minutes, it would become deep yellow due to oxidation.

acid, regained the original colour.

Reductive acetylation with zinc and acetic anhydride in presence of triethylamine.

(i) A mixture of Fuerstiaquinone (80 mg.) and zinc dust (100 mg.) was treated with acetic anhydride (1 ml.) and triethyl-:amine (1 drop), when the decolourisation took place within a minute. The mixture was quickly extracted with glacial acetic acid andthe extract was filtered under suction in a filter tube containing ca. 25 ml. of water. A white solid separated which was filtered, washed thoroughly with water and then dried in a vacuum desiccator over silica gel. The substance was very soluble in methanol, ethanol, ether, benzene, chloroform, dioxane and ethyl acetate but insoluble in ligroin. All attempts to crystallise from single solvents or solvent pairs were unsuccessful. On keeping, the white amorphous powder was found to change its colour to pale yellowish white, then yellow, yellowish brown and finally to yellowish orange. A sample, m.p. 94-98°, was sent for analysis after two weeks from the time of its preparation (Found: C,71.73; H,7.72; OAc, 19.4%. $C_{20}H_{24}O(OAc)_2$ requires C, 72.36; H, 7.53; OAc, 21.6%). Infra-red spectrum of the freshly prepared material in nujol showed bands at 3500 m, 1778 s, 1750 s, 1637 w, 1616 w, 1568 w, 1331 m, 1284 m, 1250 s, 1210 s, 1145 s, 1042 m, 1019 m, 960 m, 946 m, 889 m, 815 w, and 779 w, $cm.^{-1}$. Ultra-violet spectrum: λmax. 265 mμ and 245-49 mμ.

As the infra-red spectrum indicated the presence of free

hydroxyl group in the reductive diacetate, the latter was treated with acetic anhydride and pyridine for further acetylation. Ca. 30 mg. of the freshly prepared diacetate (white) was dissolved in 0.5 ml. of pyridine whereby a dark brown solution was obtained. On adding acetic anhydride (2 ml.) to this, the colour changed to brownish red. After the mixture had stood three days, it was poured into water and the separated solid was collected and dried. A buff coloured powder, m.p. 90-125° was obtained. This was not further investigated.

A solution of the diacetate in benzene was chromatographed on alumina. The lower pink-yellow band was eluted with benzene and the upper dark coloured band with 20% ethanol-benzene. Removal of solvents from the former fractions yeilded a yellow oil in very small amount. The other fraction gave a dark coloured amorphous solid, m.p. 73-80°. A dark coloured solution of this solid in methanol, on treatment with dil. HCl, gave a white precipitate.

A solution of the diacetate in benzene was left exposed to air for two weeks when the yellow colour gradually deepened to orange red. Benzene was removed and the red oil thus obtained was shaken with iso-octane whereby a small portion of it dissolved giving a yellow solution. The residue was then dissolved in benzene and excess ligroin was added. The precipitated solid was centrifuged and dried in vacuum desiccator over paraffin wax. A pale orange-yellow powder, m.p. 164-169°, was obtained. (Found: C, 68.19; H, 6.93).

Reductive acetylation with hydrogen (platinum) and acetic anhydride in presence of pyridine -

A solution of Fuerstiaquinone (240 mg.) in acetic anhydride (distilled, 5 ml.) was hydrogenated in presence of Adam's catalyst until the solution became completely colourless (ca. ½ hr.). Pyridine (distilled, 3 ml.) was added to this and the mixture was allowed to stand in complete absence of oxygen for four days. It was next poured into water (ca. 400 ml.) and the separated solid extracted with ether (3 x 100 ml.). (The emulsion of ether in water was broken by adding sodium chloride). The ether solution was successively washed with saturated aqueous solutions of $NaHCO_3$, $KHSO_4$ and $CdCl_2$. Finally, it was washed once more with water and then dried by leaving overnight with anhyd. Na_2SO_4. On removing the ether, a pale yellowish solid was left as the residue. This crystallised from methanol as a white solid. Recrystallisation from the same solvent gave white needles, m.p. 193-194°, 26.5 mg. On concentrating the mother-liquor, another crop of crystals (18 mg.) was obtained. Total yield was 44.5 mg. (13.2%). The substance was dried over P_2O_5 in vacuo at 100° for 3 hours. Found: C, 70.57; H, 7.77; OAc, 35.6%. $C_{20}H_{25}(OAc)_3$ requires C, 70.56; H, 7.74; OAc, 29.1%.

Optical rotation: $[\alpha]_D$ + 66° (measured with a solution of 3.6 mg. of the substance in 2 ml. of $CHCl_3$).

Ultra-violet spectrum: λmax. 282 mμ (log ϵ 2.7) and 292 mμ (log ϵ 2.8); Fig.III.

Infra-red spectrum (HCB): 2957 m, 2880 w, 1774 s, 1750 s, 1638 w, 1481 w, 1465 w, 1415 w, 1388 w, 1373 s, 1331 w, and 1288 w, $cm.^{-1}$.

Colour Reactions

An ethanolic solution of Fuerstiaquinone was hydrogenated (Pt/H_2) and the following tests were performed with the colourless solution obtained.

(i) Ferric chloride - On adding freshly prepared ferric chloride solution, a green colouration was produced.

(ii) Diazotised sulphanilic acid - Addition of p-diazobenzene sulphonic acid dissolved in aqueous sodium carbonate produced a wine-red colour.

(iii) Gibb's test[56] - Few drops of borax solution (1.91 g. $Na_2B_4O_7$, H_2O in 100 ml. water) was added and the $p_H 9$ of the solution was tested (wide range indicator paper). An aqueous suspension of 2:6-dibromo benzoquinone chloroimide was added to this, when a light green colour developed which quickly changed into dark violet colour.

Under the same condition, α-naphthol and catechol gave blue colour, but β-naphthol produced a green colour which, after some time, turned red-violet.

Reductive methylation by the action of diazomethane on the borohydride reduction product

A solution of Fuerstiaquinone in ethanol (containing a little water) was reduced with potassium borohydride in an atmos-

:phere of nitrogen and the resulting colourless solution was treated with an ethereal solution of diazomethane. No nitrogen was evolved and the mixture, on standing overnight, turned dark. On working, a dark oil was obtained which could not be purified by chromatography.

Reductive methylation by the action of diazomethane on the catalytic hydrogenation product

A solution of Fuerstiaquinone (100 mg.) in ethanol (10 ml.) was hydrogenated in presence of a platinum catalyst and the colourless solution was quickly and carefully filtered into an ethereal solution of diazomethane. Nitrogen was evolved. The mixture was allowed to stand overnight. Next day the colour of the solution remained pale yellow and after removing the excess diazomethane, ether was boiled off. A very viscous brown oil was obtained. It was dissolved in benzene and chromatographed on alumina. A fraction was collected on eluting with 40% chloroform-benzene, and removal of solvents from this yielded a very pale yellow glass. This was chromatographed once again on alumina (20% chloroform-benzene) and the filtrate obtained was freed from solvents. An almost colourless glass was obtained which upon standing solidified to an almost white powder, m.p. 65-68°. The substance could not be crystallised. It was easily soluble in methanol, ethanol, and benzene but completely insoluble in ligroin. Analysis of the amorphous powder gave 16.28% OCH_3. $C_{20}H_{26}O(OCH_3)_2$ requires 18.02% OCH_3.

Infra-red spectrum (nujol): 3341 b, 1703 w, 1642 m, 1594 m, 1236 s, 1229 s, 1158 s, 1118 m, 1026 m, 1012 s, 959 m, 882 w, and 811 w $cm.^{-1}$.

Deuteration of Fuerstiaquinone

4 ml. of carbon tetrachloride* was added to 1 ml. of an 1% solution of Fuerstiaquinone in carbon tetrachloride (used before in the determination of infra-red spectra, see page 82) and the diluted solution was taken in a glass stoppered test-tube (10 ml., Quickfit). 4-5 drops of heavy water (containing 95% D_2O) was added to this and the tightly stoppered test tube was shaken for 3 hours. Anhydrous sodium sulphate (freshly dehydrated) was then added to the test tube and the mixture was agitated overnight. The solution was then filtered and the filtrate was distilled (to ensure complete removal of water) under reduced pressure until the volume of the solution had reduced to ca. 1 ml. Infra-red spectrum was determined with this solution. A lithium fluoride prism and the same cell thick-:ness as before (see page 82) was used. No OH-absorption was pres-:ent in the spectrum.

Infra-red spectrum: 3008 m, 2965 s, 2950 s, 2920 s, 2906 s, 2859 m, 2846 m, 1456 m, 1441 m, 1388 m, 1362 m, 1348 s, 1335 s, 1279 s, 1168 m, 1149 w, 1107 w, 1067 m, and 1054 m $cm.^{-1}$.

Methylene anthrone[33]

Anthrone (10 g., m.p. 149-51°), ethanol (100 ml.), liquor

* Carbon tetrachloride was distilled before use and the middle fraction was collected.

formaldehyde (5 ml.) and piperidine (4-5 drops) were refluxed for half an hour. The yellow solution, on cooling, yielded methylene anthrone, m.p. 144° (twice recrystallised from ethanol). Infra-red spectrum (nujol): 1636 s, 1586 s, 1308 s, 1210 s, 1167 s, 1094 m, 1075 s, 1023 m, 964 m, 930 s, 917 s, 886 m, 870 m, 808 w, and 777 s $cm.^{-1}$.

Attempted reactions with 2:4-dinitrophenylhydrazine and hydroxylamine

(i) 2:4-dinitrophenylhydrazine (250 mg.) was dissolved in concentrated sulphuric acid (0.5 ml.) and alcohol (3.5 ml.) was added. A solution of Fuerstiaquinone (ca. 50 mg.) in ethanol (1 ml.) was treated with a few drops of the reagent when a blue colour was formed which quickly disappeared producing an almost colourless solution. No crystalline material separated on stand-:ing or on warming the mixture on the water-bath (at 60-70°) for half an hour. When diluted with water, the acid rearrangement product of Fuerstiaquinone separated in the form of a white turbidity.

The D.N.P. derivative could not be obtained by warming an ethanolic solution (ca. 3 ml.) of Fuerstiaquinone (60 mg.) and 2:4-dinitrophenylhydrazine (50 mg.) on the water-bath for two hours under reflux.

(ii) An ethanolic solution of Fuerstiaquinone and hydroxyl-:amine (excess) were mixed and warmed. The red colour was dis-:charged and no crystalline material was obtained.

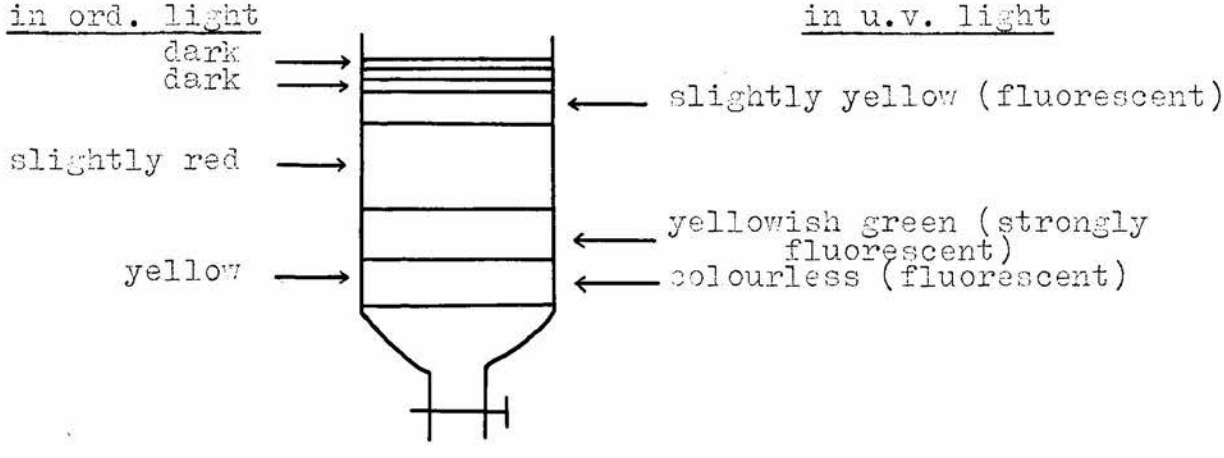
Hydroxylamine hydrochloride (250 mg.) and sodium acetate

(ca. 250 mg.) were dissolved in water (ca. 1 ml.) and an ethanolic solution (ca. 2 ml.) of Fuerstiaquinone (100 mg.) was added. The mixture was warmed on thewater-bath but, on cooling, no crystalline material separated. When it was diluted with water, Fuerstia-:quinone was returned.

Zinc dust distillation

(a) small scale - One end of a glass tubing (6 x 0.6 cm.) was drawn out into a capillary and the root of the capillary was stuffed loosely with some asbestos. The tubing was filled in the following order with zinc dust, Fuerstiaquinone + zinc dust (1:100 mixture), and zinc dust, to make layers of 1 cm. (a), 0.5 cm. (b), and 0.5 cm. (c) respectively and the tube was sealed outside layer (c). After first heating (a) slightly with a micro-flame, the tubing was heated from (c) to (a) and (a) to (c) by swinging the flame backward and forward. During the course of distillation, care was taken to distil with the heat transmitted through the zinc layer and to move the flame slowly towards new portions of the tube containing the yet undecomposed material (the red liquid absorbed on zinc dust goes colourless on decomposing). Forty of these distillations were carried out on a mixture of 200 mg. of Fuerstiaquinone and 20 g. of zinc dust (Analar). In each case a slightly greenish yellow oil distilled at the root of the capillary and a greenish sublimate deposited at the sealed end of the tube. The tubes were cut into halves and, after removing the heated mixture from inside, they were

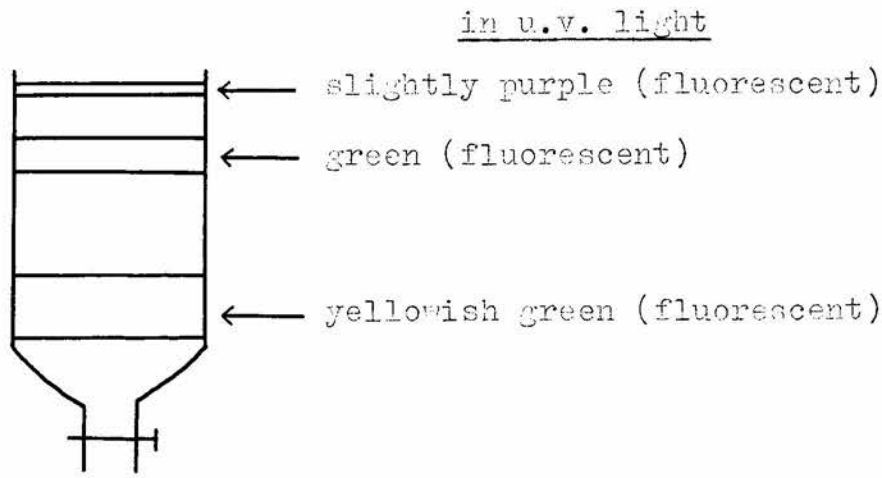
thoroughly washed with ligroin. The ligroin solutions (yellow with green fluorescence) were combined, filtered, concentrated and then chromatographed on alumina. On eluting with ligroin, a dark band (decomposed materials) remained sticking at the top and a yellow band moved very slowly, above which there was a slightly red band. Ligroin containing increasing amounts of benzene was used and with 50% benzene-ligroin, a greenish yellow, strongly fluorescent band (in u.v. light) separated from the top. The yellow bands were eluted with pure benzene and the eluates were evaporated, leaving brown, impure oily residues. These were dissolved in benzene and the combined solution again chromato-:graphed on alumina, using benzene for elution. The chromatogram is described below:



Three fractions were collected (on eluting the three bands shown by arrows on the right side of the chromatogram).

(i) The middle fraction, on evaporation, left a small amount of a greenish yellow oil. This was dissolved in benzene

and again chromatographed on a thin alumina column. The chroma-:togram is described below:



The two lower bands were eluted with benzene and collected in fractions. The first fraction, on evaporation, gave a yellowish green glass which did not crystallise. Its ultra-violet spectrum showed maxima at 230, 257, 325, 361 and 405 mμ (Fig.V). By slow evaporation of an alcoholic solution of this compound, some pale orange coloured solid was obtained which did not melt but decom-:posed at about 360°. The second fraction, which was a colourless solution, gave small amount of a white solid on removal of benzene. This crystallised from ethanol (by slow evaporation) in the form of long, shining, colourless plates, m.p. 103° (sharp).
Ultra-violet spectrum: λmax. 225-230 and 255-260 mμ (Fig.VI).

(ii) The first fraction, on evaporation, gave a dark brown oil which was left aside. After 2-3 months it was noticed that a solid had crystallised out from the oily residue. The crystals were carefully freed from the adhering impurities by washing with methanol. These were beautiful, colourless plates, m.p. 215-18°.

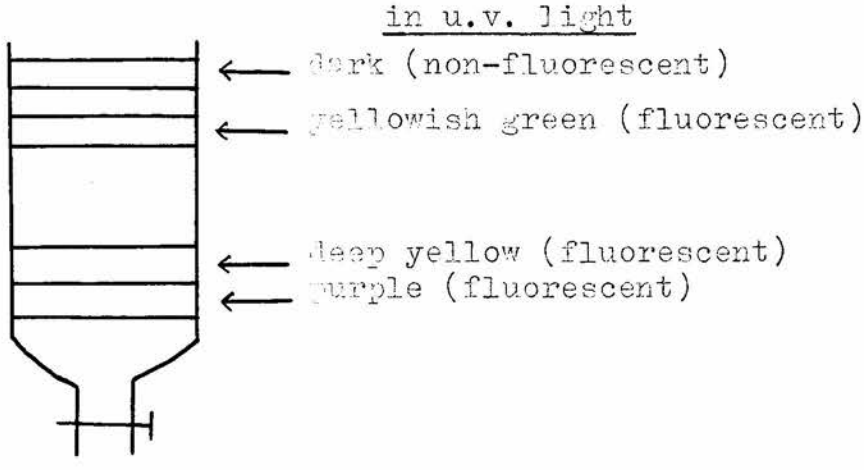
The solid was found to sublime at atmospheric pressure.

(iii) The last fraction gave a yellow oil which was dissolved in ethanol. On slow evaporation, small amount of a golden yellow solid, m.p. 115-20°, was obtained.

None of the substances obtained in this distillation were in sufficient amount to permit an analysis. Therefore, the zinc dust distillation was repeated with a greater quantity of Fuerstia-:quinone. But this time a somewhat different result was obtained. This is described below.

(b) big scale - One hundred and seventy seven distillations were carried out on a mixture of 1.2 g. of Fuerstiaquinone and 120 g. of zinc dust. The tubes, drawn at one end into capillary, were filled up, sealed and heated by means of a micro-flame as described before. At first, on slight heating, a colourless oil distilled over which condensed in the capillary as a white solid. When strongly heated afterwards, a greenish yellow oil was found to collect in droplets at the root of the capillary. The capillar-:ies were cut and washed with benzene. The tubes were also cut into halves and, after pouring out the heated mixture, were washed with benzene. The benzene solutions were combined and then chromato-:graphed on alumina. The fluorescent bands (purple, yellow and yellowish green) were washed off with benzene leaving behind a dark band of the decomposed materials sticking at the top of the column. The eluate was yellow with green fluorescence. The heated zinc dust mixtures were collected together and thoroughly extracted with

benzene whereby a very dark solution was obtained. This was chromatographed twice on alumina and the clean eluate (yellow benzene solution fluorescing green) of the fluorescent bands were combined with that obtained before. The combined solution was chromatographed on alumina using benzene for elution. The bands appeared on the chromatogram as shown below.



The separation between the two lower bands was not good and so their eluates were collected in one fraction. The second fraction of the top fluorescent band, on evaporation, gave a greenish brown oil (C) which seemed impure. After evaporating benzene, the residue from the former fraction was dissolved in ligroin and again chromatographed on alumina. Elution with ligroin gave a good separation this time. The purple and yellow fluorescent bands were eluted with 10% benzene-ligroin and 80% benzene-ligroin respectively; in the former case a colourless eluate was obtained which, on evaporation, gave a colourless oil (A) and in the latter case the eluate produced a yellow-red oil (B), a solution of which

on being slowly evaporated gave an amorphous orange-red solid.

The substance (C) was purified by chromatographing twice on alumina. The green (top) and the colourless (below) fluorescent bands were eluted with benzene. The coloured eluate, on evapor-:ation, left a green oil which did not crystallise. On adding ethanol, an orange coloured amorphous solid separated which did not melt even at 325° but decomposed (decomp. starts at 230-40°). Sublimation under high vacuum was not successful and the substance did not form a picrate or a 2:4:7-trinitrofluorenone complex. It showed absorption maxima at 230, 257, 325, 361 and 405 mμ. The absorption spectrum (Fig. V) could not be identified with that of any known aromatic hydrocarbon. No maximum was found between 450-600 mμ. The colourless eluate gave very small amount of a colourless oil whose spectrum (Fig.VII) showed only benzenoid absorption: $\lambda_{max.}$ at 225-230 and 255-260 mμ (cf. Fig.VI). The substance (B) was dissolved in benzene and chromatographed on alumina using ligroin as the eluting agent. The main greenish yellow band was eluted with 80% benzene-ligroin after washing off the lowest thin colourless-fluorescent band with 10% benzene-ligroin. The coloured eluate was evaporated and the solution of the residue in ligroin (in which it was difficultly soluble) was again chroma-:tographed on a thin column of alumina. This time a pale yellow band (dark in u.v. light) separated which was eluted with 50% benzene-ligroin. The eluate (yellow solution with weak green fluorescence) on evaporation, left a yellow oil which did not

crystallise. Its absorption spectrum (Fig. VIII) showed maxima at 245 and 345 mμ.

The colourless oil (A, 146 mg.) was the chief product of this distillation. It had a kerosine-like smell and, on adding alcohol, it changed into a white flocculent solid. It was almost insoluble in methanol, moderately soluble in ethanol, soluble in ether and highly soluble in ligroin and benzene. On cooling hot saturated solutions in ethanol or iso-propyl alcohol, the substance separated in the form of a white flocculent mass. Attempts to crystallise from ethanol-ether, ethanol-ligroin and methanol-ether (by cooling or slow evaporation) were unsuccessful. It seemed that the substance was low-melting (m.p. ca. 40-50°). On very slow evaporation of an ethanolic solution, some long, shining, colourless crystalline plates were obtained but these were contaminated with the oil drops and so the melting point could not be determined. It did not form a picrate. Its ultra-violet absorption spectrum (Fig. IV) showed maxima at 228 and 273 mμ and minimum at 253 mμ.

It was considered that the oil might be a mixture of stereoisomers. It was dissolved in ligroin and chromatographed twice on alumina. The purple fluorescent band was eluted first time with 5% benzene-ligroin and then with 20% benzene-ligroin. The eluate was evaporated and a solution of the residue in ethanol was very slowly evaporated. The same result was obtained as before, i.e. few crystalline plates mixed with oil. The nature of the ultra-violet spectrum and the low-melting point suggested

that the molecule was partly aromatic (only benzene residue). So dehydrogenation with chloranil was attempted to effect complete aromatisation.

Dehydrogenation with chloranil[52]

2:3:5:6-tetrachloro 1:4-benzoquinone (chloranil) was recrystallised from glacial acetic acid, washed with a mixture of alcohol and ether and then dried.

Sulphur free xylene was refluxed with sodium and then distilled.

The oil (127 mg.), chloranil (850 mg., 5.1 mole per mole of the donor on the basis of $C_{14}H_{18}$) and xylene (5 ml.) was gently refluxed for forty hours in an atmosphere of nitrogen. The colour of the solution changed from brownish yellow to dark. After cooling, the solution was diluted with ligroin (ca. 50 ml.) and the precipitated solids were removed by filtration. The filtrate was chromatographed thrice on alumina column (8" x ½") so that the eluate of the fluorescent band was completely colour-:less. (Elution was done with ligroin). The eluate, on evapor-:ation, gave a colourless semi-solid mass (like low-melting wax), 64 mg. Properties of this substance were almost similar to those of the non-dehydrogenated oil. It was insoluble in methanol and ethanol and did not crystallise from usual solvents. It did not form a picrate or 2:4:7-trinitrofluorenone complex. On heating at about 50-60°, it melted to a thin oil. Sublimation under high vacuum was attempted but without success. Ultra-violet spectrum

(Fig. IX) of the substance in cyclo-hexane showed maximum at 224 mμ ($E^{1\%}_{1\ cm.}$ 90) only. Analysis of a sample (washed with hot ethanol and then kept at 50° in vacuo over silica gel for 24 hours) gave: C, 84.82; H, 13.34; Mol.wt. (camphor), 382.

Oxidation with alkaline hydrogen peroxide

(i) A solution of Fuerstiaquinone (275 mg.) in ethanol (ca. 5 ml.) and hydrogen peroxide (100 vol., ca. 1 ml.) were mixed and the mixture treated with dil. NaOH solution (1-2 drops). Upon addition of alkali, the red colour changed to purple which quickly disappeared producing a colourless solution. This was left to stand overnight. Then the solution was made just acidic (litmus) with dil. H_2SO_4 and poured into water (ca. 150 ml.). The aqueous mixture was extracted with ether (2 x 100, 1 x 50 ml.) and the ether solution was allowed to stand over anhydrous Na_2SO_4 for about 15 hours to ensure complete dryness. The filtered solution was then freed from the solvent whereby a very pale yellow oil mixed with some needle-shaped crystals were obtained. This was warmed (70-80°) with a small amount of benzene in which the crystalline solid and a small portion of the oil dissolved leaving behind the major portion of the oil. The benzene solution was removed but it did not yield any crystal on concentrating or cooling. Only slow evaporation in air produced crystals which were filtered, washed few times with benzene and then dried in vacuo over paraffin wax. Thick colourless prisms, m.p. 222-23°, 29.6 mg. Found: C, 77.03; H, 8.01; C-CH_3, 7.77%.

$C_{14}H_{18}O_2$ requires C, 77.03; H, 8.31; 1C-CH_3, 6.88%.

The crystalline compound was found to be very soluble in chloroform, ether and dioxane, moderately soluble in methanol and ethanol, slightly soluble in benzene and insoluble in ligroin and water. The substance when dissolved in a solvent by heating, does not crystallise on cooling the solution. Crystals could be obtained by slow evaporation only. Solutions of the substance were found to fluoresce in ultra-violet light producing the same colour as produced by a solution of the colourless hydrocarbon obtained from the zinc dust distillation. The solid sublimed at ca. 178° and 105° under normal and 0.3 mm. pressure respectively. The sublimate (crystallised from ethanol by slow evaporation) had m.p. 223°.

Analysis gave: C, 76.97; H, 8.28.

Ultra-violet spectrum: $\lambda_{max.}$ 280 mμ (log ε 2.9) and 236 mμ (log ε 3.9); $\lambda_{min.}$ 271 mμ; Fig.X.

Infra-red spectrum (nujol): 2654 w, 1675 s, 1601 s, 1570 m, 1411 s, 1341 m, 1310 s, 1294 s, 1280 s, 1261 s, 1196 s, 1176 m, 1163 s, 1143 m, 1124 m, 1074 m, 1033 m, 1000 m, 989 m, 943 s, 899 m, 859 w, 842 s, 785 m, 750 m, 729 m, and 720 m $cm.^{-1}$.

(a) Colour reactions - The compound, $C_{14}H_{18}O_2$, did not respond to the Fluorescein test. Its alcoholic solutions also did not produce any colouration on treatment with aqueous solutions of diazotised sulphanilic acid (made slightly alkaline) or ferric chloride.

(b) Potentiometric titration - A prepared solution of sodium

hydroxide (ca. 200 mg.) in water (500 ml.) was standardised by titrating potentiometrically* a solution of oxalic acid (3.3 mg., $C_2H_2O_4,2H_2O$, Analar). Phenylacetic acid (5.7 mg.) was dissolved in water and the solution was similarly titrated with the standard alkali solution (.0102N). The end-point (4.17 ml.) gave value for the molecular weight of the substance as 134 (actual mol. wt. 136). The p_H value at the half-neutralisation point was found to be 4.6 (lit. p_K value at 25° is 4.3). Next an ethanolic solution of the compound, $C_{14}H_{18}O_2$ (4.6 mg.), was titrated with the same alkali solution. In this case the molecular weight was found to be 215 (mol. wt. of $C_{14}H_{18}O_2$ is 218). The end-point was at 2.09 ml. and the p_H value at the half neutralisation point was 5.7.

The oil that was left after extracting the oxidation product with benzene was next investigated. This oil was found to become more and more viscous and deep yellow in colour on standing. It did not contain any easily volatile constituent as, on heating to 100° under reduced pressure (ca. 11 mm.), nothing could be dis-:tilled. At about 120° a yellow viscous oil mixed with some sticky solid distilled off, which could not be purified. Attempted sublimation under high vacuum always gave a yellowish-brown, very sticky semi-solid mass. Once the distillate (sticky semi-solid), obtained by heating at ca. 200° under 2 mm. pressure, was dissolved in ether and the solution, on evaporation in air, gave a few very fine, long, colourless crystalline needles embedded in a viscous

* Pye p_H meter was used. Nitrogen was bubbled through the solution of the acid. Readings of p_H were plotted against the volume of alkali added and the end-point was determined from the inflexion of the curve.

yellow oil. But these might be of the $C_{14}H_{18}O_2$ compound.

(c) Paper chromatography[72] The oil was examined by chroma-:tography in the following solvent systems in attempt to obtain maximum resolution.

(1) EtOH (80 ml.)-H_2O (15 ml.)-Conc.NH_4OH (3 ml.diluted to 5 ml.)

(2) EtOH (60) - H_2O (40) - Conc. NH_4OH (3)

(3) EtOH (80 ml.) - Conc. NH_4OH (3 ml. diluted to 5 ml.)

(4) n-PrOH (7) - H_2O (3)

(5) n-PrOH (9) - H_2O (1)

(6) n-PrOH (1) - H_2O (1)

(7) n-BuOH (half saturated with H_2O)

(8) n-BuOH (saturated with H_2O)

(9) n-PrOH (6) - Conc. NH_4OH (3) - H_2O (1)

(10) n-PrOH (7) - H_2O (2) - dil. HCl (1)

(11) C_6H_6 (8) - AcOH (8) - H_2O (1)

(12) n-BuOH (95) - HCOOH (5) - saturated with H_2O

(13) n-BuOH saturated with 1.5N NH_4OH

The spots were detected by the ultra-violet photographic technique. In the case of solvents (5), (6), (7), (8), (9) and (12), no resolution was effected but only single spots appeared with R_f 0.60, 0.75, 0.86, 0.55, 0.70 and 0.79 respectively. Streaking was found to occur in solvents (2), (3), (4), (10) and (11) and the respective R_f were 0.42-0.64, 0.54-0.78, 0.17-0.78, 0.69-0.93 and 0.74-0.90. Solvent (1) was effective for partial resolution in which case a spot and a streak with R_f 0.48 and 0.65-0.82 were produced (R_f of $C_{14}H_{18}O_2$ marker was 0.81). Solvent (13)

was found to be the best. It produced three widely separated spots with R_f 0.74,* 0.22 and 0.0 (R_f of $C_{14}H_{18}O_2$ marker was 0.78). These chromatograms were dried and then sprayed separately with (i) KI-KIO_3 -starch,[73] (ii)** ethanolic bromocresol green followed by lead acetate[74] and (iii) aqueous-alcoholic diazotised sulphanilic acid (made slightly alkaline). In the first and second cases, three blue and three yellow spots appeared proving that they were all acidic (the spot with R_f 0.0 seemed to be the most acidic). In the third case, no coloured spot appeared.

The wide difference in R_f values suggested the possibility of separation of the acid mixture on a cellulose column using n-butanol saturated with 1.5N NH_4OH for elution.

Separation on cellulose column[75]

Ca. 250 mg. of Fuerstiaquinone was oxidised with alk. H_2O_2 and the acid $C_{14}H_{18}O_2$, was crystallised out from the oxidation mixture. The residual oil was mixed*** with about 5 ml. of n-butanol (saturated with 1.5N NH_4OH) and the mixture was soaked on the top of a cellulose column (10" x 1.2"). Elution was continued with the solvent mentioned and the eluates were collected in 10 ml. fractions (about fifty). The presence of each component in the eluate was detected by paper chromatography and appropriate tubes were combined. Two main fractions were thus obtained. On evapor-

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* This spot was not composite but slightly streaky. It is now understood that it was actually due to two substances ($C_{14}H_{18}O_2$ and another acid).

** (i) seemed to be a better reagent for detection than (ii)

*** A portion of the oil does not dissolve in this solvent.

:ation, the first fraction gave a very viscous oil mixed with some solid and the second fraction gave only the oil. Paper chromatography of these produced single spots in each case (R_f 0.72 and 0.22). By slow evaporation of an ethanolic solution of the residue obtained from the first fraction, it was possible to obtain a small amount of a crystalline material (colourless prisms) but this seemed to be the acid, $C_{14}H_{18}O_2$. The residue from the second fraction could not be crystallised. The top of the cellu-:lose column was taken out and eluted with ethanol but the eluate did not yield any crystalline material.

It was considered that better purification might be effected on the esters of these acids (see below).

(ii) A solution of Fuerstiaquinone (1.35 g.) in ethanol (ca. 15 ml.) and hydrogen peroxide (100 vol., ca. 5 ml.) were mixed and dil. NaOH solution was added dropwise until the mixture went colourless with the separation of a white solid. It was allowed to stand overnight. On acidification with dil. H_2SO_4, the white solid was found to dissolve with effervescence. The solution was poured into water (ca. 500 ml.) and the mixture extracted 3-4 times with ether. The ethereal solution was completely dried by leaving over anhydrous Na_2SO_4 for a long time and then it was evaporated. Colourless needles mixed with a very pale yellow oil were obtained. A small amount of ethanol (ca. 0.5 ml.) was added to this and then cooled in the refrigerator to complete crystall-:isation. The crystals were filtered, washed 2-3 times with ice-

cooled ethanol and then dried in vacuo over silica gel. Colour-:less, hexagonal prismatic needles,* m.p. 223°, yield 112 mg.

(a) Optical activity - A solution of the crystalline substance ($C_{14}H_{18}O_2$, 120.4 mg.) in chloroform (10 ml.) did not show any optical rotation when a polarimeter tube of 1 dm. length was used.

(b) Methyl ester of $C_{14}H_{18}O_2$ - An ethereal solution of the acid (110 mg.) was treated with an excess of ethereal diazo-:methane. Nitrogen gas was evolved and the mixture was left to stand overnight. Then diazomethane and ether were boiled off, leaving a residue which was extracted with hot ligroin. The filtered ligroin solution was concentrated to about 1 ml. and then allowed to evaporate slowly in air. The methyl ester crys-:tallised out in the form of thin colourless plates, m.p. 81°, yield 113 mg.

Ultra-violet spectrum: $\lambda_{max.}$ 281 mμ (log ε 3.0) and 243 mμ (log ε 4.1); λ min. 270 mμ; Fig.XI.

Infra-red spectrum (Nujol): 1711 s, 1684 m, 1626 w, 1593 m, 1566 w, 1538 w, 1309 m, 1294 s, 1275 s, 1250 s, 1202 s, 1182 s, 1149 s, 1076 s, 1038 s, 971 m, 960 m, 893 m, 861 w, 837 m, 804 w, 788 s, and 783 s $cm.^{-1}$.

Dehydrogenation of the methyl ester of $C_{14}H_{18}O_2$ with chloranil[52]

2:3:5:6- tetrachloro 1:4-benzoquinone (chloranil) was

* During melting point determination the substance seemed to change its crystalline shape at 200-204°.

recrystallised from boiling glacial acetic acid. The crystals were washed with a mixture of alcohol and ether and then dried.

Sulphur free xylene was refluxed with sodium for one hour and then distilled.

The methyl ester (112 mg.), chloranil (250 mg. i.e., 2.1 mole per mole of the ester) and xylene (5 ml.) were gently refluxed for twenty hours in an atmosphere of nitrogen. The colour of the solution changed from brownish yellow to dark brown. After cooling, the solution was diluted with ligroin (25 ml.) whereby the quinones separated out. These were filtered and extracted by boiling with ligroin. The ligroin solutions were combined and then chromatographed on alumina column (8" x ½"). The fluorescent band (in ultra-violet) was eluted with 5% benzene-ligroin and the eluate, on evaporating, left a pale yellow oil which soon crys-:tallised. The crystals were dissolved in ligroin and the solution was chromatographed once again on alumina in the same way. Removal of solvents from the eluate left a residue which crystallised from ligroin by slow evaporation in the form of long colourless plates, m.p. 81°, 67.2 mg. Found: C, 78.59, 78.83; H, 8.31, 8.48; C-CH_3, 6.15%, 5.54%. $C_{15}H_{20}O_2$ requires C, 77.59; H, 8.62; IC-CH_3, 6.46%. X-ray powder photography was done with this material and the methyl ester of $C_{14}H_{18}O_2$. Exactly the same pattern was ob-:tained in both the cases proving that the two substances were identical.

The oil (obtained from the oxidation of 1.35 g. of Fuerstia-

:quinone) was methylated with diazomethane and the product (1.32 g.) was subjected to distillation under reduced pressure. At about 80° and under 11 mm. pressure, a colourless fragrant smelling liquid was collected as the distillate. It had n_D^{20} 1.4224. Com-:bustion analysis gave: C, 35.36; H, 8.37.

Infra-red spectrum: 3380 b, 2993 s, 2948 s, 2920 m, 2894 m, 1716 s, 1651 w, 1594 w, 1542 w, 1453 s, 1429 s, 1386 s, 1335 m, 1279 m, 1186 m, 1127 s, 1090 s, 1047 s, 948 m, 882 w, 842 w, and 776 w $cm.^{-1}$.

The small quantity of the distillate indicated that it was the smallest component of the oxidation mixture. Presumably, it was the ester of the acid whose R_f was found to be 0 in the paper chromatographic examination (solvent system 13).

On heating the residue at 126-140° under 0.25 mm. pressure, a crystalline solid mixed with some yellow oil sublimed. This was dissolved in ligroin, and then chromatographed on alumina. The lower yellowish band (in u.v.) was eluted with 20% benzene-ligroin and the eluate, on evaporation, gave a very small amount of a crystalline solid, m.p. 80-81°. The upper band (fluorescing pale blue in u.v. light) was eluted with 40% benzene-ligroin and the eluate was evaporated, leaving a residue which crystallised from ligroin in the form of long, thin, colourless plates, m.p. 81°, 32 mg. Found: C, 77.35; H, 8.38. Calc. for $C_{15}H_{20}O_2$: C, 77.58; H, 8.62. Therefore this substance was the methyl ester of $C_{14}H_{18}O_2$.

The semi-solid residue, left after sublimation, was dissolved in benzene and the solution was chromatographed on alumina using benzene as the eluting agent. Three bands were found to separate i.e., two yellow bands above a pale blue fluorescent band (u.v.). The first fraction of the eluate, on removal of benzene, gave a very small amount of the methyl ester of $C_{14}H_{18}O_2$ (m.p. 80-81°). The second fraction (middle yellow band), on evaporation, gave a very pale yellow glass (A). This was easily soluble in alcohol and ether but completely insoluble in ligroin. The topmost band was eluted with 20% methanol-benzene and the solvent was removed, leaving a brownish yellow oil (B). This was easily soluble in alcohol but not in ether. On adding a small amount of the latter solvent, a brownish white solid was found to separate from the oil; but it dissolved in excess of the solvent.

A solution of (A) in ethanol was decolourised by boiling with charcoal and the residue, left after evaporating off the ethanol, was dissolved in benzene. This was chromatographed on alumina. Two bands separated of which the lower one fluoresced pale green and the upper one colourless in the ultra-violet light. Both the bands were eluted with benzene. The eluate from the green fluorescent band, on evaporation, left a very pale yellow glass which turned into almost solid (157 mg.) on being completely freed from benzene (in vacuum over paraffin wax).
Ultra-violet spectrum: $\lambda_{max.}$ at 278 mμ ($E_{1\,cm.}^{1\%}$ 49.5) and 246 mμ ($E_{1\,cm.}^{1\%}$ 75); Fig.XII.

Infra-red spectrum: 3458 m, 2953 s, 2887 s, 1750 s, 1683 s, 1649 s, 1465 s, 1445 s, 1390 s, 1341 s, 1288 s, 1261 s, 1225 s, 1204 s, 1165 s, 1121 s, 1081 s, 1029 s, 996 s, 931 m, 894 m, 877 m, 852 m, 840 m, 812 m, and 773 m $cm.^{-1}$.

An alcoholic solution of this substance did not give any colouration with aq. $FeCl_3$. The eluate from the colourless fluorescent band, on evaporation and subsequent standing in vacuo over paraffin wax, gave a colourless glass (41 mg.) which was embedded with some white crystals. This was considered to be a mixture of (A) and (B).

The brownish yellow oil (B) was dissolved in ethanol and decolourised by boiling with charcoal. The colourless oil, on treatment with a small amount of ether, gave a white solid. This was dissolved in excess of ether and the solution was evaporated. The residue was a white amorphous solid (230 mg.). It was soluble in methanol, benzene, chloroform, ethyl acetate and dioxane but insoluble in ligroin. It could not be crystallised. An alcoholic solution of this substance did not produce any colouration with aq. $FeCl_3$.

Ultra-violet spectrum: $\lambda_{max.}$ 235-240 mμ ($E^{1\%}_{1\ cm.}$ 117); Fig. XIII.

Infra-red spectrum: 3440 b, 2948 , 2882, 1730, 1677, 1648, 1456, 1385, 1269, 1175, 1141, 1087, 1033, 987, 919, 898, 883 $cm.^{-1}$. All bands are strong.

Oxidation with hydrogen peroxide in presence of ferrous sulphate

Hydrogen peroxide (100 vol., ca. 1 ml.) was added to a

solution of Fuerstiaquinone (20 mg.) in ethanol (2-3 ml.) and some crystals of ferrous sulphate was dropped in the mixture. Oxidation was allowed to proceed until the red colour was discharged (with occasional addition of hydrogen peroxide and ethanol). The solution was then evaporated to dryness and the residue extracted with anhydrous ether. On removing the ether, a brown oil was obtained which could not be purified by chromatography.

Oxidation with nitric acid

(i) Conc. nitric acid (5 ml.) was added to Fuerstiaquinone (100 mg.) when a vigorous reaction took place with the evolution of much heat and gas. After the reaction had subsided, the solution was heated on the water bath (at 80°) for 4-5 hours and then kept in a vacuum desiccator over caustic potash until all the nitric acid was removed. The residue was treated with some water and again dried over caustic potash.

Fluorescein test with a portion of the dried material gave positive result (intense green fluorescence) indicating the pres-:ence of a dicarboxylic acid or acid anhydride. The oxidation product was then chromatographed on paper using solvent systems, ethanol (80 ml.)/water (15 ml.)/Conc. ammonia (3 ml. diluted to 5 ml.) and n-butanol saturated with 1.5 N NH_4OH, and the dried chromatograms were photographed in the ultra-violet light. In the former case, a streak (R_f 0.53-0.83) and in the latter, four spots and a streak (R_f 0.76, 0.66, 0.53, 0.28 and 0.0-0.19) were observed. The oxidation product was found to be easily soluble

in methanol and water (aqueous solution did not give any precipit-:ate with silver nitrate or barium chloride). An aqueous solution was filtered and evaporated to dryness. The residue crystallised from ethanol (charcoal) in the form of very small, colourless, octahedral prisms which, on heating upto 350°, did not melt but slightly decomposed. The small amount of the crystalline material did not permit analysis.

(ii) Conc. nitric acid (5 ml.) was cooled in ice and Fuerstiaquinone, (785 mg.) was added to it in small portions. The quinone, on being oxidised with the evolution of heat and gas, slowly went into solution. The temperature of the solution was not allowed to rise above room temperature. When all the substance had dissolved, the solution (red) was heated on the water bath (80-90°) for one hour and then kept in a vacuum desi-:ccator over caustic potash in order to remove nitric acid. On drying a very viscous, red oil was obtained. This was treated with little water and again dried over caustic potash.

A portion of the oxidation product was slowly heated under high vacuum (.05 mm.) but nothing sublimed except that the sub-:stance charred when the temperature rose to ca. 150°. The oxid-:ation product was found to be completely soluble in a small amount of methanol or ethanol in the cold. Attempts to crystallise it from different solvents were not successful. Upon standing for about eight weeks, the viscous red oil was found to yield some crystals which were carefully freed from adhering impurities by

washing with ether. The crystals were beautiful, shining, colour-:less prisms, m.p. 205° (decomp.). It gave positive Fluorescein test. On heating, it started to lose water at 75° (when the crystals turned milky-white, most probably due to the formation of the anhydride) and to decompose at 170°. Analysis of the sub-:stance (after drying *in vacuo* over P_2O_5 at 35° for 16 hours) gave the figures: C, 20.89; H, 3.70; N, 6.34.

After removing the crystalline material, the above ethereal solution (which contained the remaining portion of the oxidation product) was treated with diazomethane. Nitrogen was evolved and on working up, the methylated material was obtained as a pale yellow glass. This was insoluble in ligroin but soluble in benzene. A benzene solution was chromatographed on alumina but no crystalline material was isolated.

Oxidation with potassium permanganate

Aqueous potassium permanganate solution (1%) was slowly added to a solution of Fuerstiaquinone (*ca.* 200 mg.) in ethanol (10 ml.) until the colour of the permanganate persisted. The mixture was then acidified, extracted with ether and the extract, after being washed with water, was dried (Na_2SO_4). On removing the solvent, a yellow oil (smell resembling that of cyclohexanol) was obtained which gradually changed into a very sticky semi-solid mass. This substance did not respond to the Fluorescein test. Paper chromatographic examination was made as in the case of nitric acid oxidation product; four ultra-violet light absorbing

spots (R_f 0.86, 0.63, 0.57 and 0.17) were found on the chromato-:gram that was run in n-butanol saturated with 1.5 N NH_4OH. In the case of ethanol (80 ml.)/water (15 ml.)/conc. ammonia (3 ml. diluted to 5 ml.), a long streak (R_f 0.89-0.70) was obtained. The oxidation product was easily soluble in ethanol and completely insoluble in ligroin. When treated with methanol, a small amount of a white crystalline solid was found to separate; but this was not investigated further.

Ozonolysis

A stream of ozonised oxygen (containing 2% ozone) was passed through a solution of Fuerstiaquinone (175 mg.) in carbon tetrachloride (20 ml.) until complete decolourisation took place (ca. 15 min.) along with the separation of a white solid (ozonide). The ozonised product was mixed with water (20 ml.) and the mixture was distilled by heating on the water bath (near boiling point). The end of the condenser was kept immersed in distilled water taken in a conical flask. When all the carbon tetrachloride had distilled over (½ hour) heating was stopped and the decomposed ozonide (which appeared as dark brown oil drops floating on water) was extracted with ether. (The distillate did not respond to the chromotropic acid test for formaldehyde.[76] In a previous experiment the ozonide was decomposed by steam distillation and the distillate, in that case, did not contain any formaldehyde but was faintly acidic to litmus.) The ethereal extract, after drying (Na_2SO_4) was evaporated, leaving a brownish-yellow oil.

The aqueous phase, that remained after extracting with ether, was found to be strongly acidic to litmus and it also gave a white precipitate with $AgNO_3$. No precipitate or colouration was produced with $BaCl_2$ and $FeCl_3$. The aqueous solution was con-:centrated (ca. 5 ml.) under reduced pressure and then chromato-:graphed on paper using the solvent, n-butanol saturated with 1.5N NH_4OH. A photograph of the chromatogram showed only one u.v. light absorbing spot (R_f 0). On spraying with KI-KIO_3-starch reagent, the paper chromatogram revealed a blue spot (R_f 0). The concentrated aqueous solution of the acid was next evaporated to dryness (desiccator) when a small amount of colourless needles with yellow oily contaminants were obtained. These were insoluble in chloroform, benzene, ligroin, acetone, ethyl acetate and dioxane and slightly soluble in ether. Recrystallisation was possible from methanol and the colourless micro-crystals obtained were found not to possess a characteristic melting point because at the stage of melting it changed into another substance with the evolution of CO_2. The acid started to melt at 110° with decompos-:ition and completed at 160°. (Care should be taken while crystall-:ising the acid because, if it is heated for this purpose to a high temperature some change takes place, presumably what is described, producing a substance which is insoluble in water, ethanol and methanol). The brownish yellow oil (from ether extract) was examined by paper chromatography. Photograph of the chromatogram run in n-butanol saturated with 1.5 N NH_4OH, dis-

:closed three ultra-violet absorbing spots (R_f 0.94, 0.61 and 0.21). The oil did not give the Fluorescein test. It seemed to form a semicarbazone derivative.

Semicarbazone: 0.5 g. of semicarbazide hydrochloride was dissolved in 2 ml. of a saturated aqueous solution of sodium acetate and the reagent was mixed with a solution of the oil in 2 ml. of methanol.[77] No crystalline material separated on standing for an hour. So few drops of methanol were added to clear the solution and then it was left to stand overnight. On adding water, a solid was precipitated which seemed to be the desired derivative but it could not be crystallised from methanol or acetic acid.

Ultra-violet spectrum of Fuerstiaquinone

Determination of spectrum with an ethanolic solution of Fuerstiaquinone (6.87 mg. per litre) gave a maximum at 435 mμ ($\log \epsilon$ 4.07) and an inflexion at 260 mμ ($\log \epsilon$ 3.4). This solution was preserved and its spectrum again determined after one year and nine months (colour of the solution remaining yellow as before). This time an entirely different type of absorption was noticed. Two maxima appeared at 330-335 mμ ($\log \epsilon$ 3.7) and 250 mμ ($\log \epsilon$ 4.04); Fig.XV.

Ultra-violet Spectra

On the following pages are the ultra-violet spectra, which have been referred to in the last two sections. All spectra were determined in purified ethanol solution (p.81) except one (Fig. IX) which was determined in cyclohexane (spectroscopic grade).

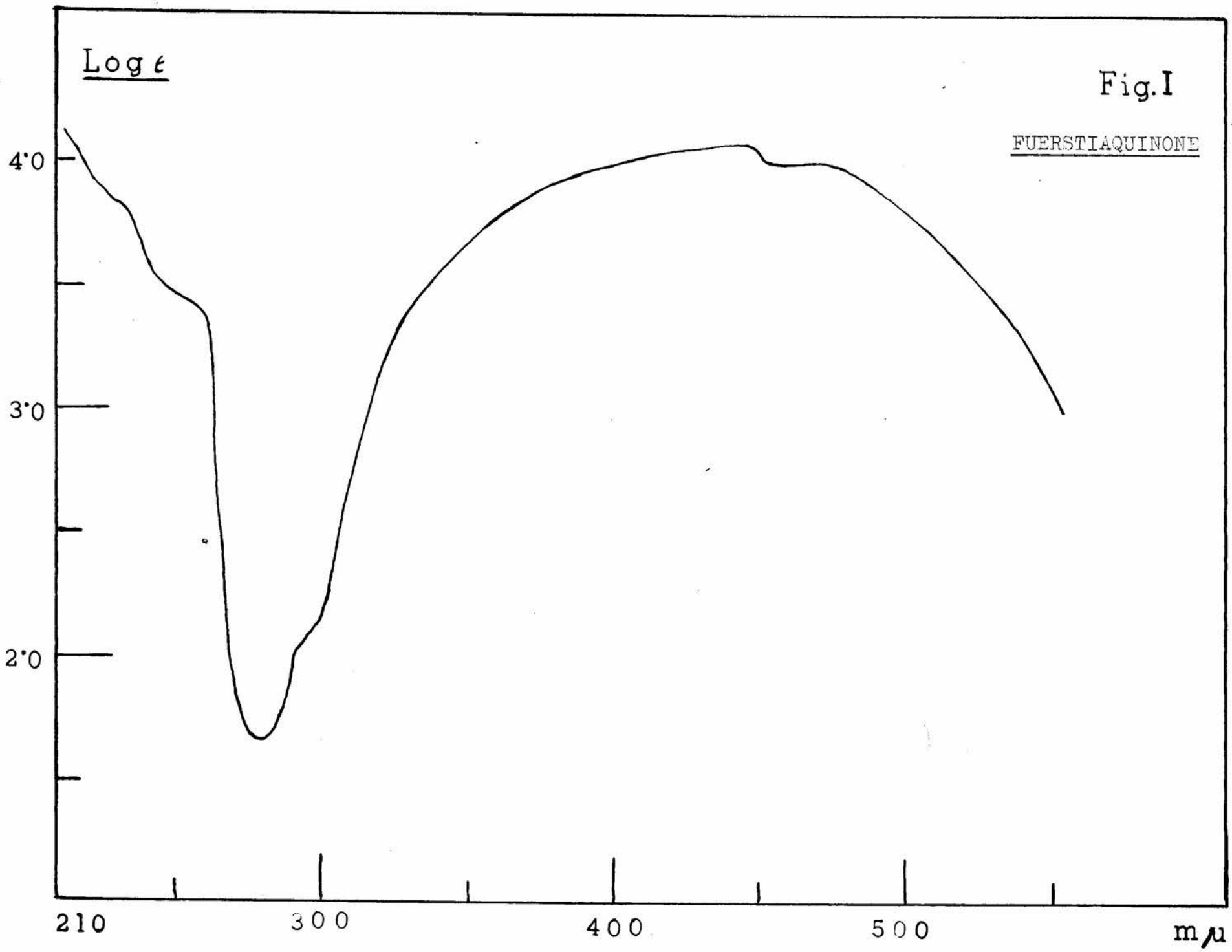


Fig.I

FUERSTIAQUINONE

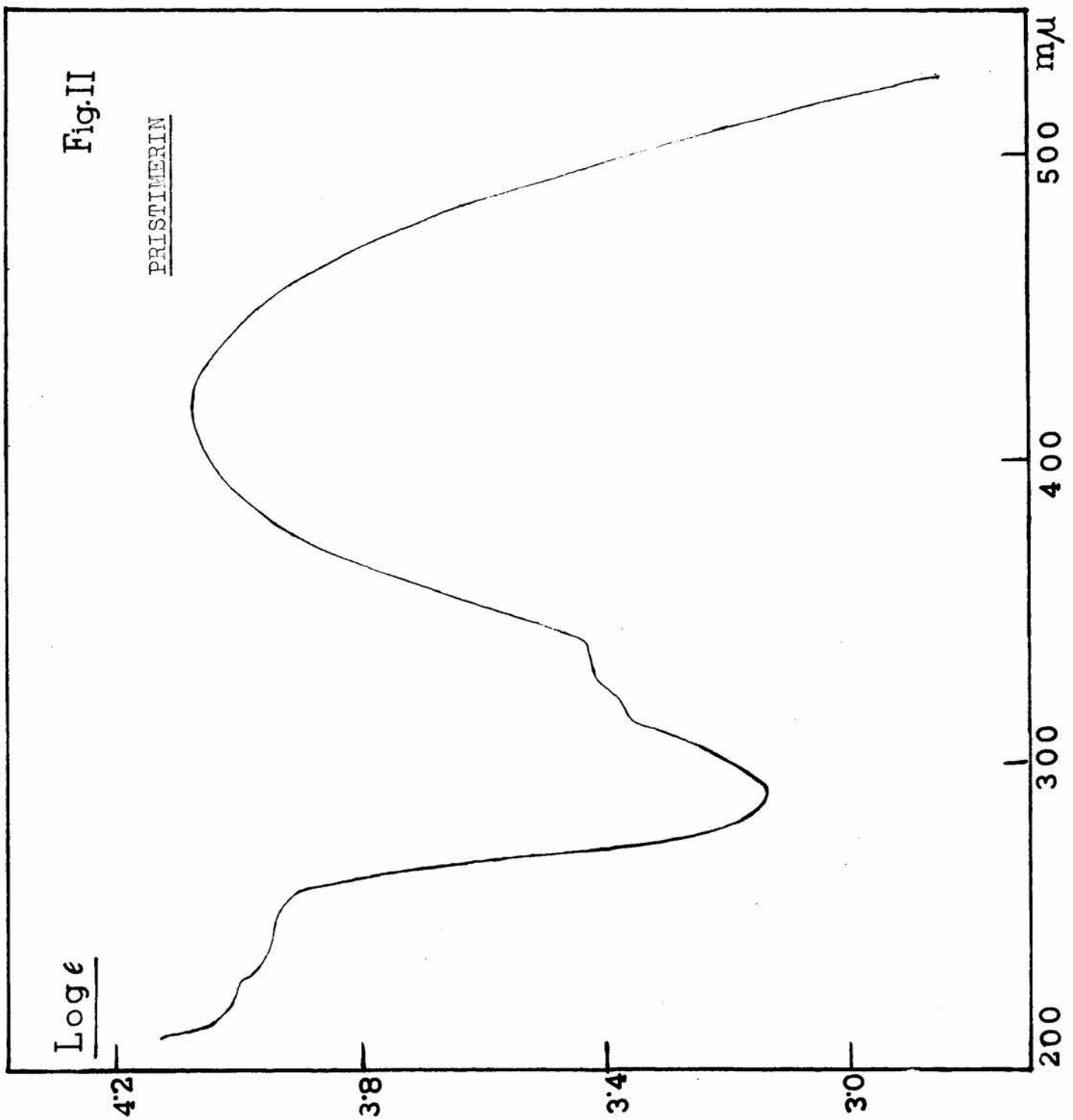
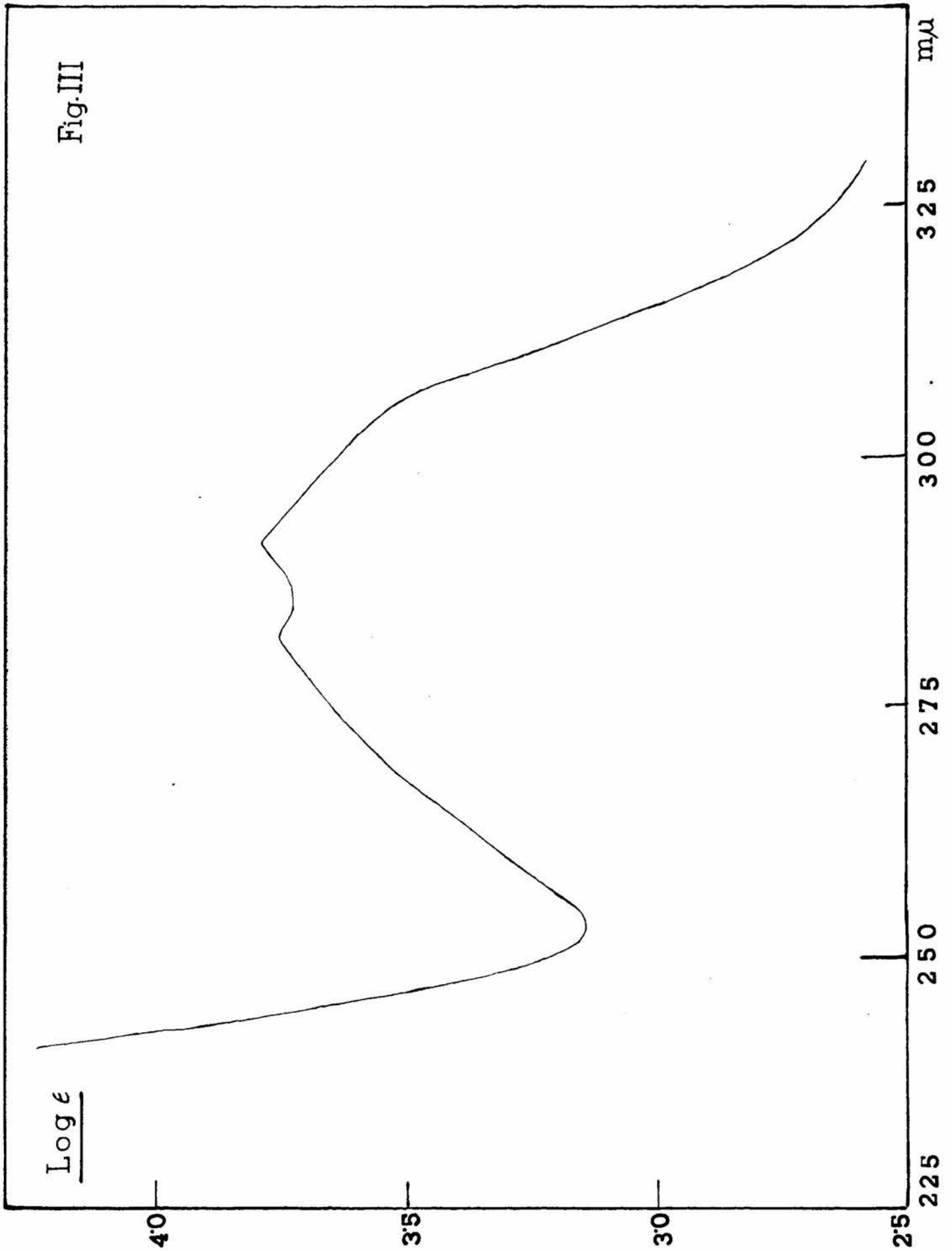


Fig.II
PRISTIMERIN
Log ε
4·2
3·8
3·4
3·0
200
300
400
500
mμ

Fig.III



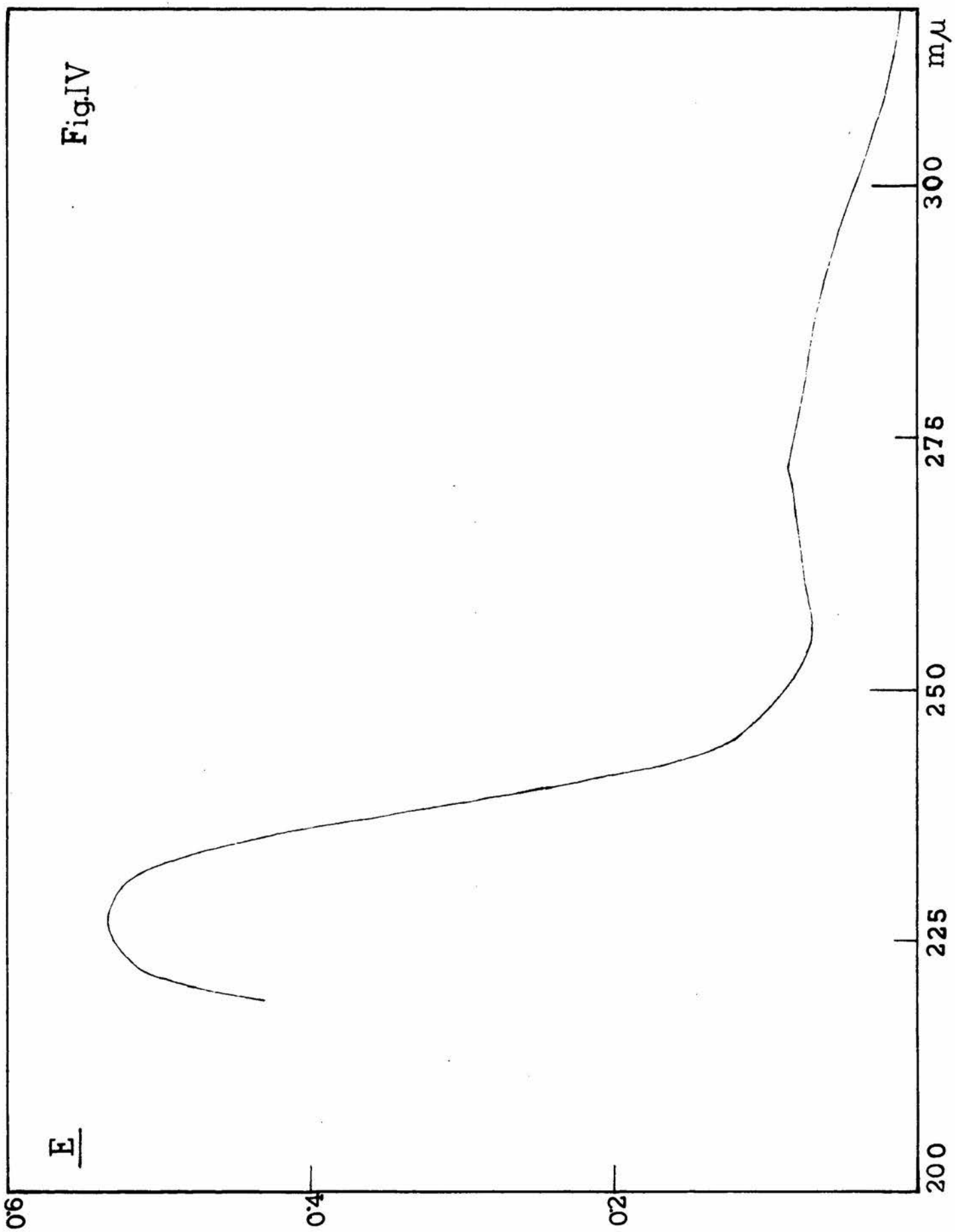


Fig.IV
E
0.6
0.4
0.2
200
225
250
275
300
mμ

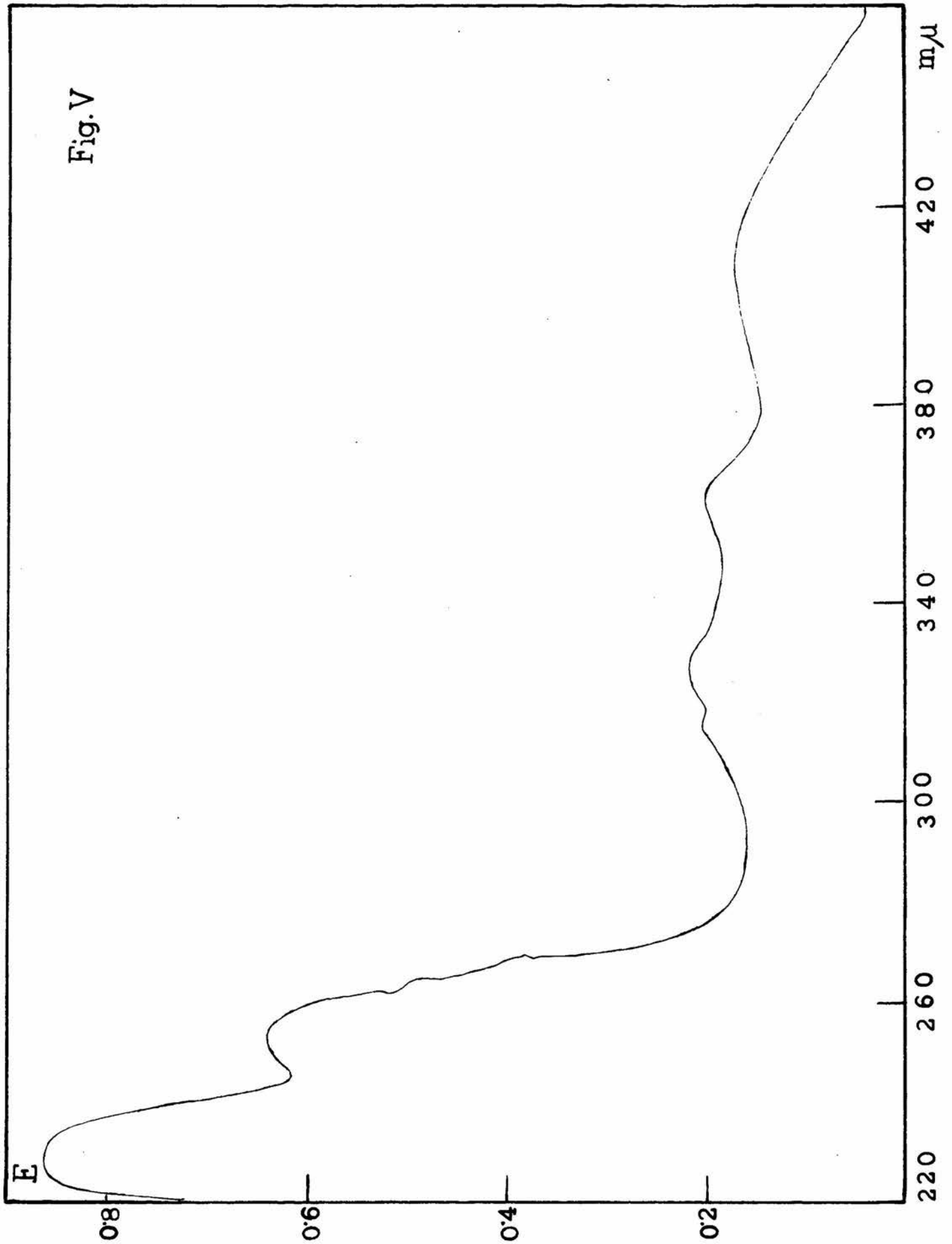


Fig. V
E
0·8
0·6
0·4
0·2
220
260
300
340
380
420
mμ

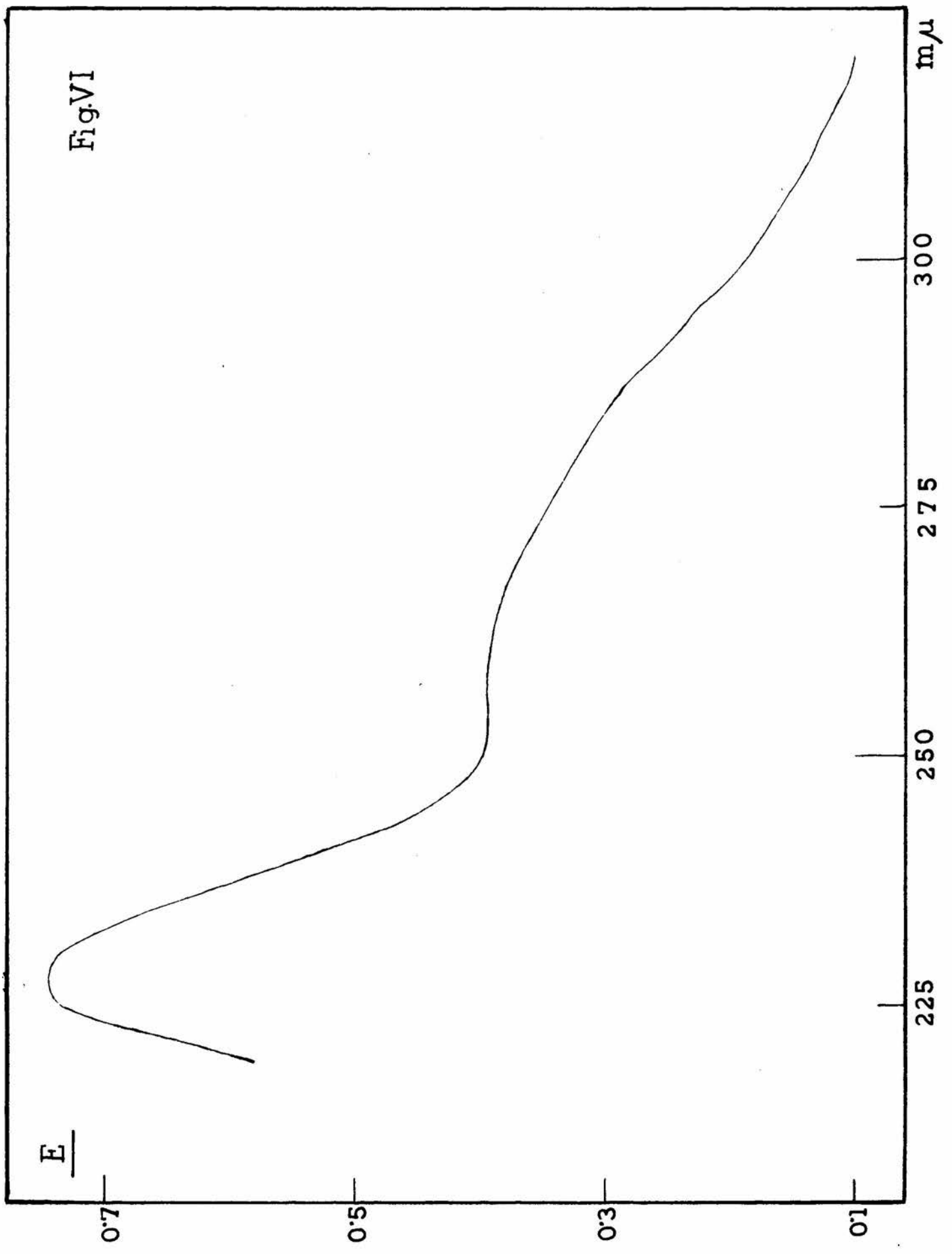


Fig.VI
E
0·7
0·5
0·3
0·1
225
250
275
300
mμ

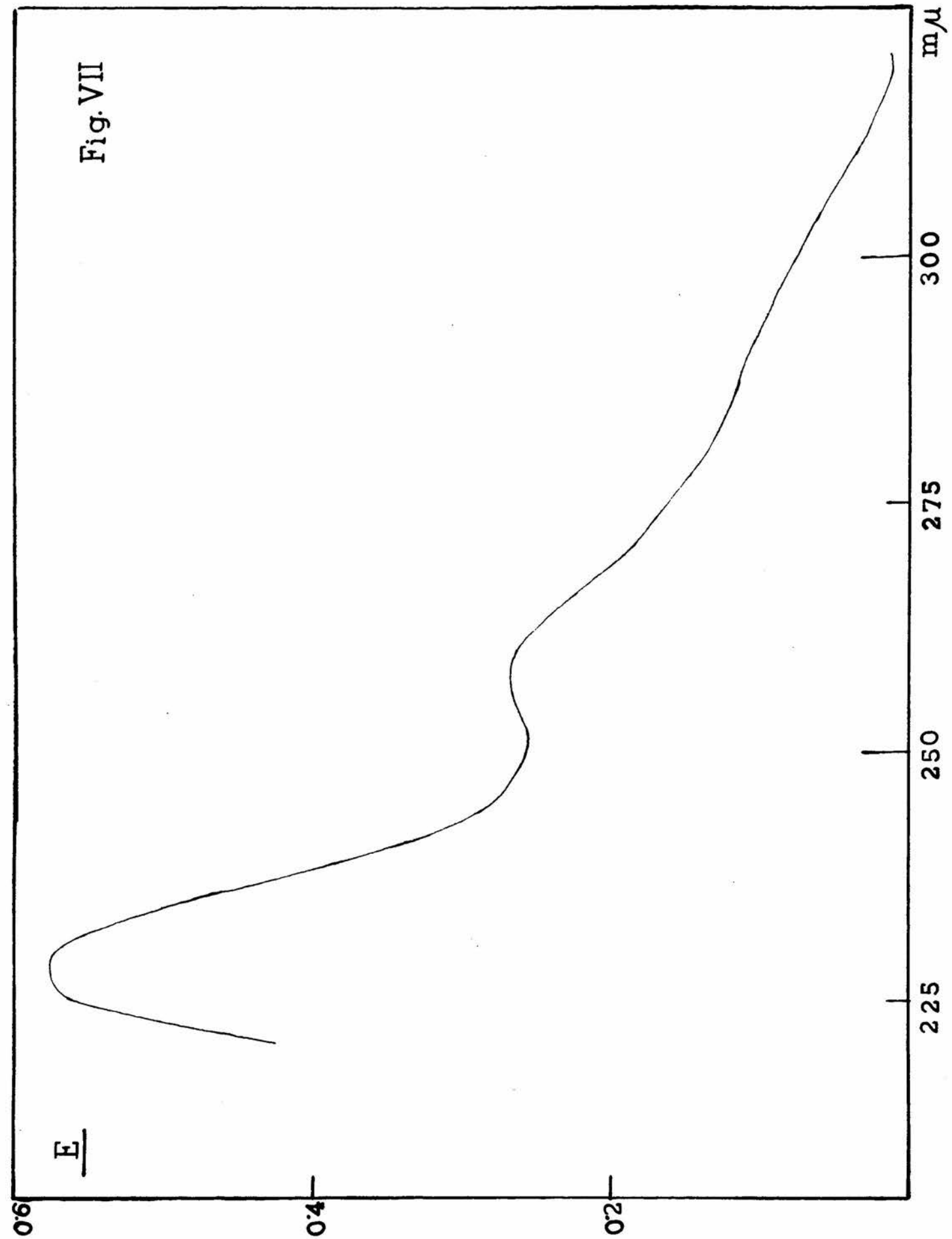
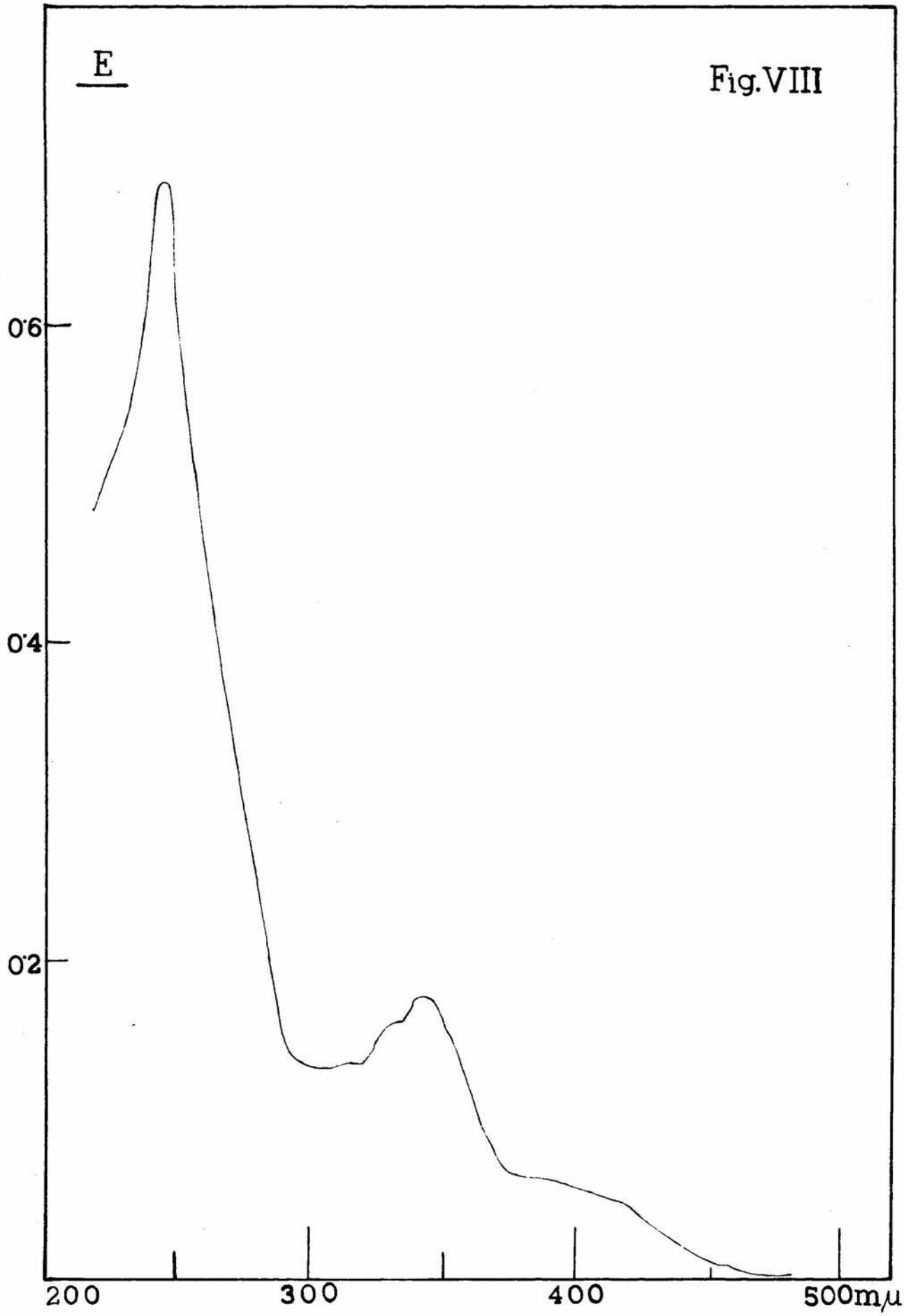


Fig. VII
E
0·6
0·4
0·2
225
250
275
300
mμ


E
Fig.VIII
0·6
0·4
0·2
200
300
400
500mμ

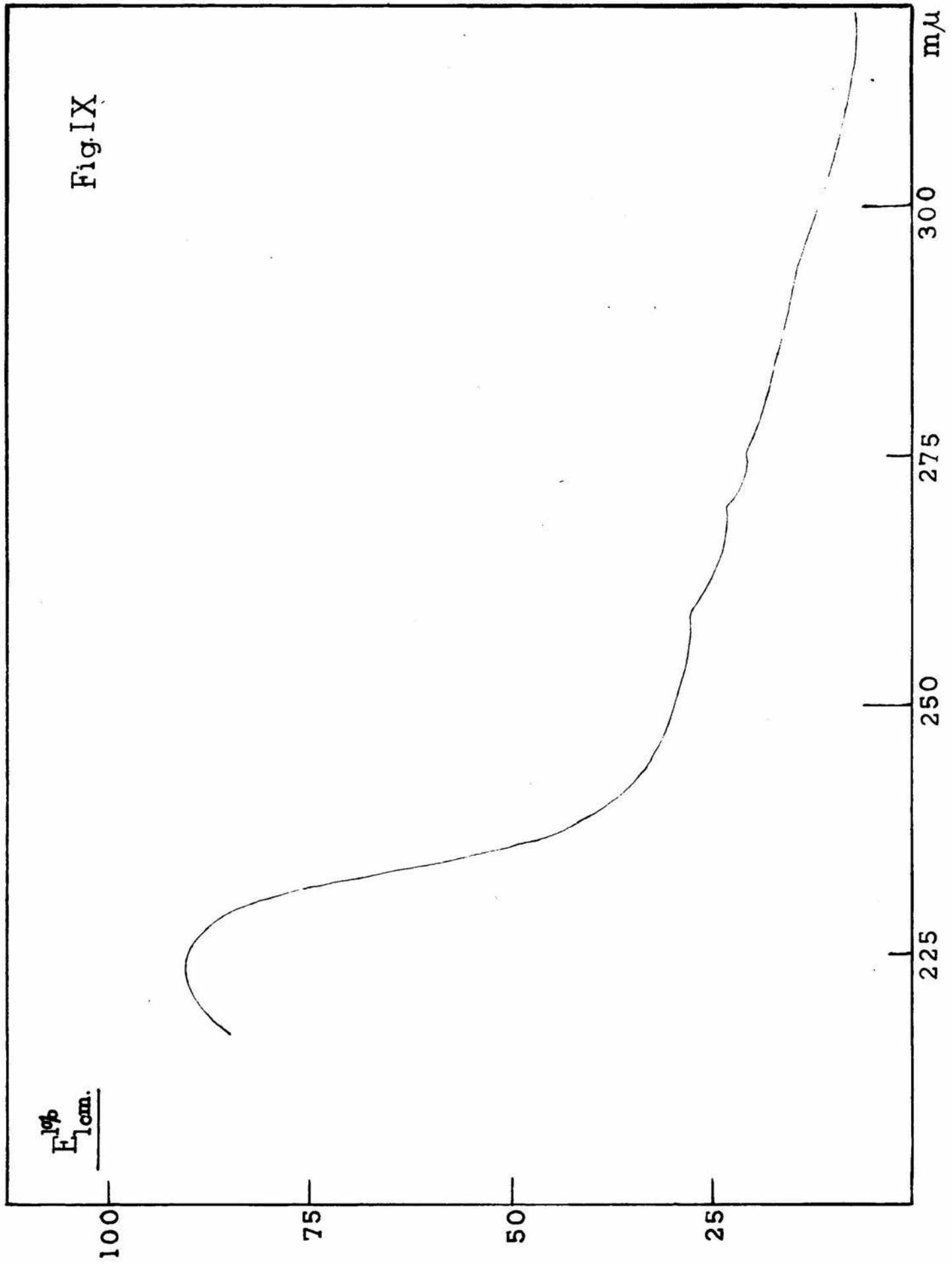
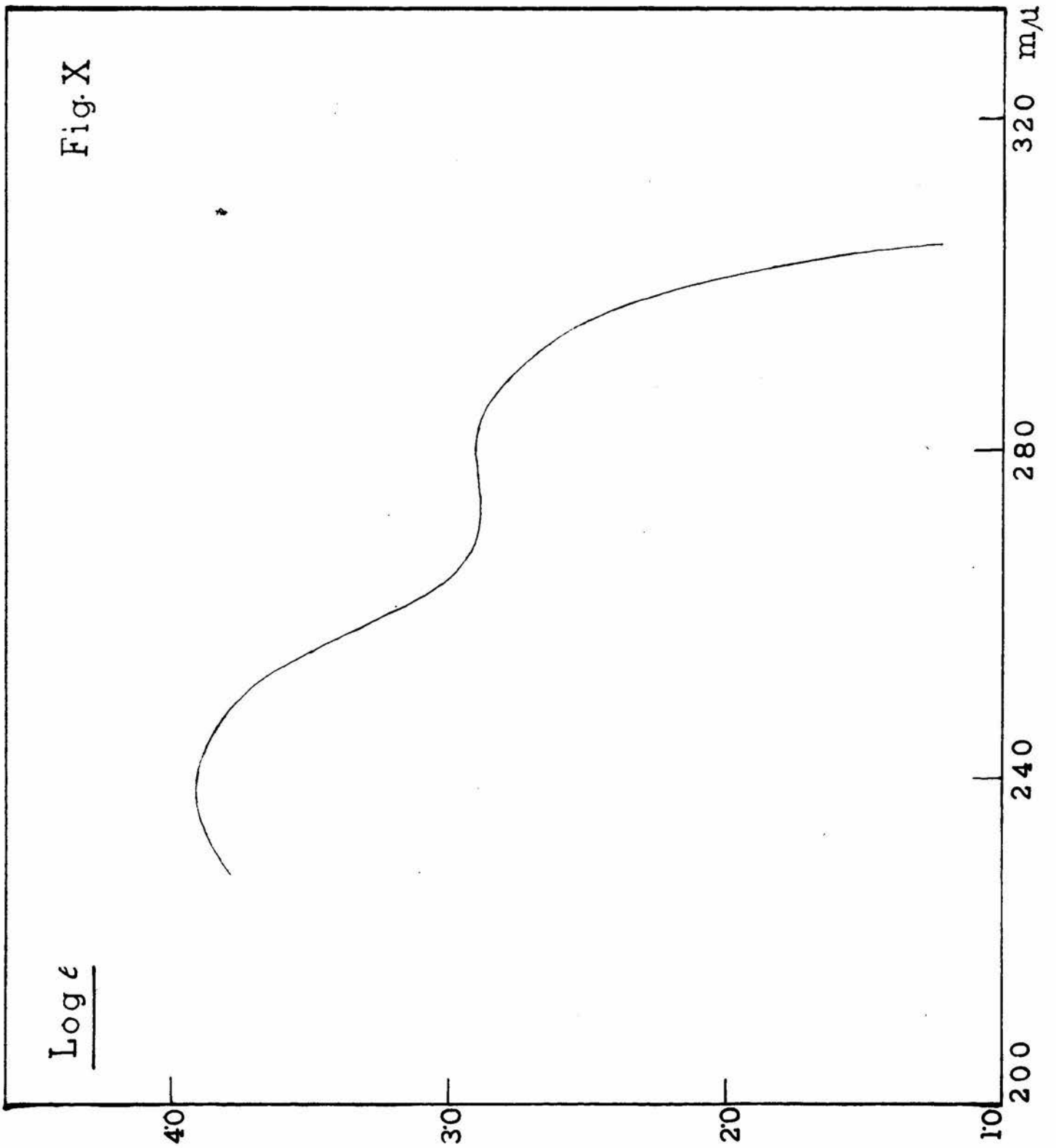
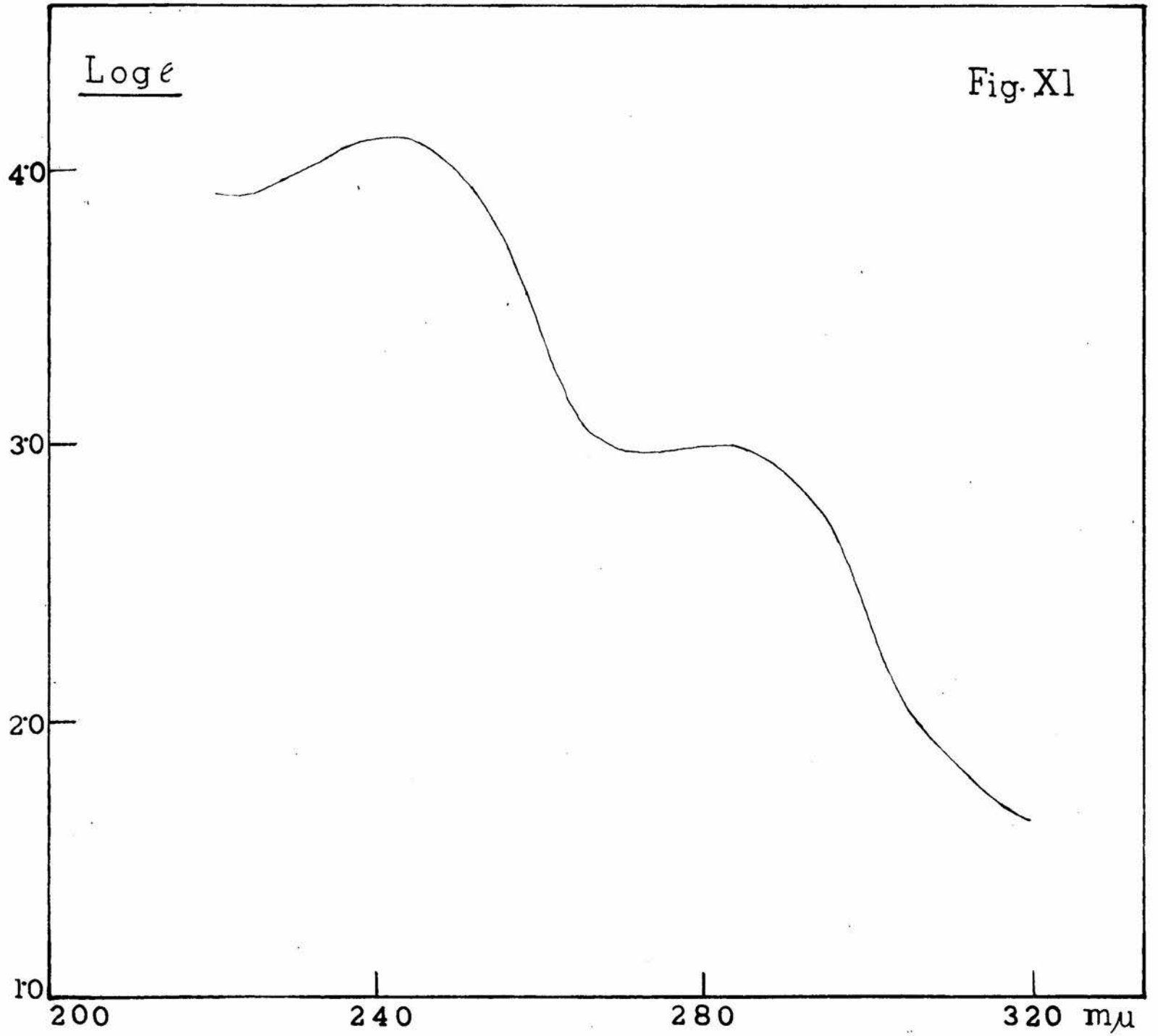


Fig.IX
$E^{1\%}_{1cm.}$
100
75
50
25
225
250
275
300
mμ


Fig·X
Log ε
4·0
3·0
2·0
1·0
200
240
280
320
mμ


Log ε
Fig. XI
4·0
3·0
2·0
1·0
200
240
280
320 mμ

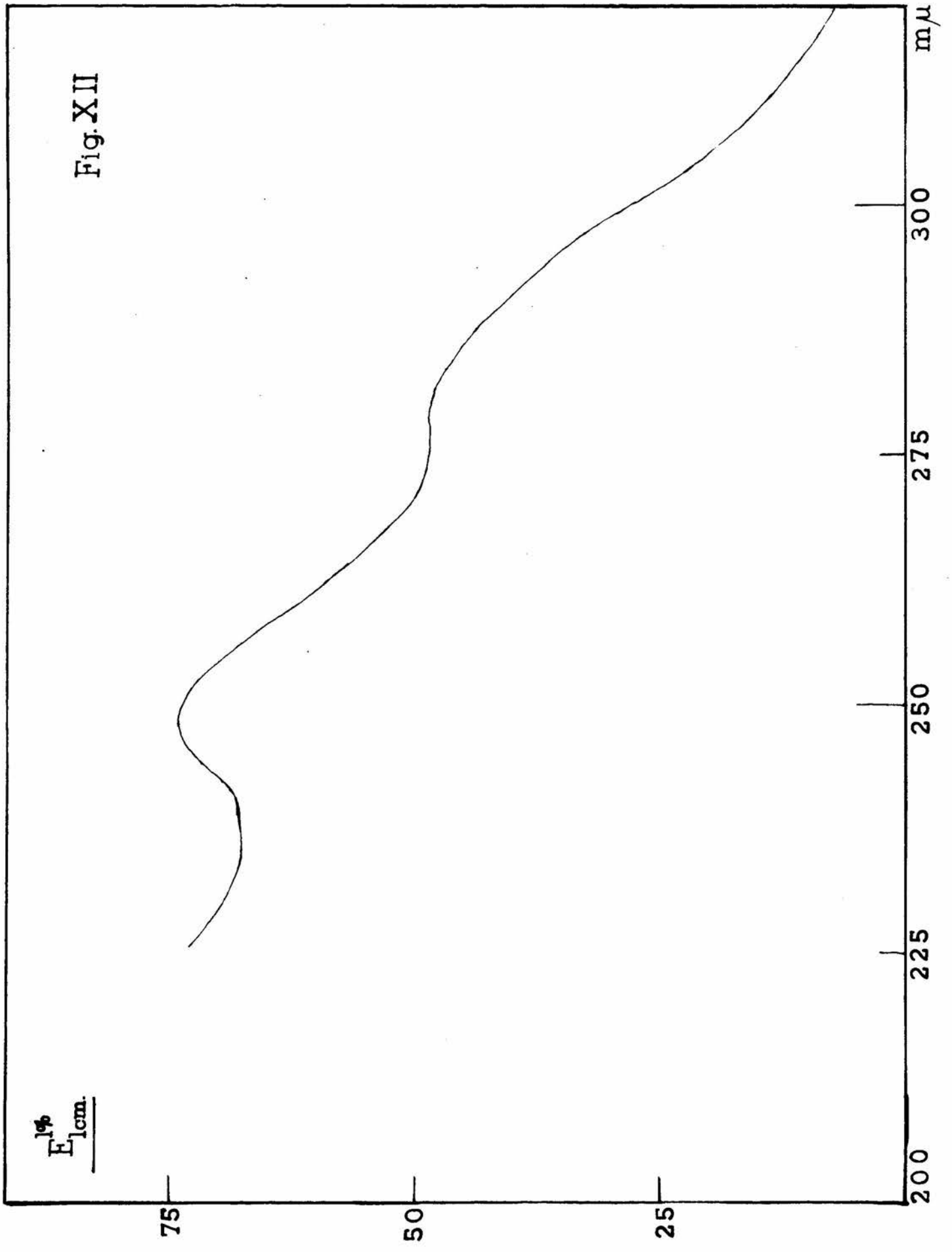


Fig. XII
$E^{1\%}_{1cm.}$
75
50
25
200
225
250
275
300
mμ

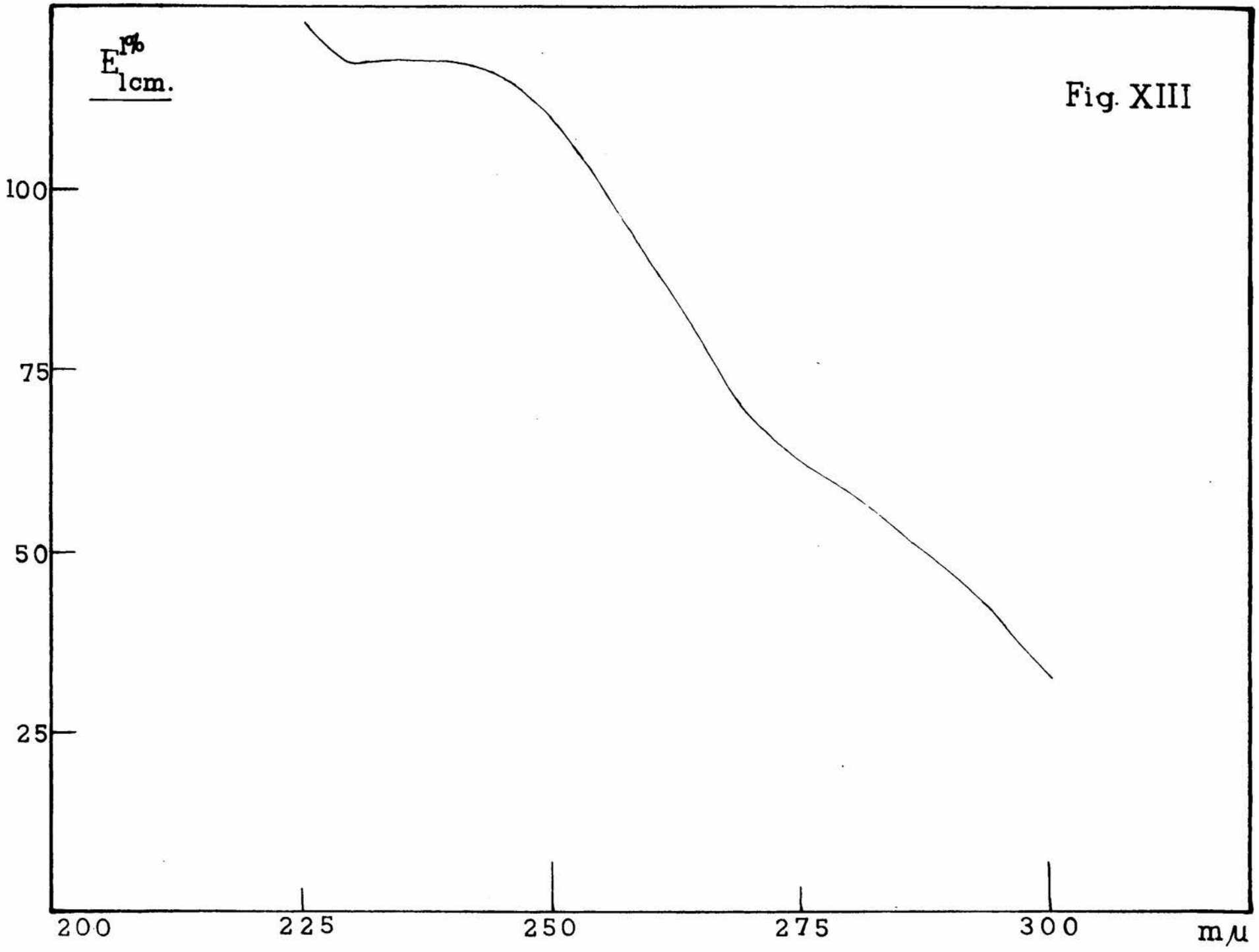


Fig. XIII

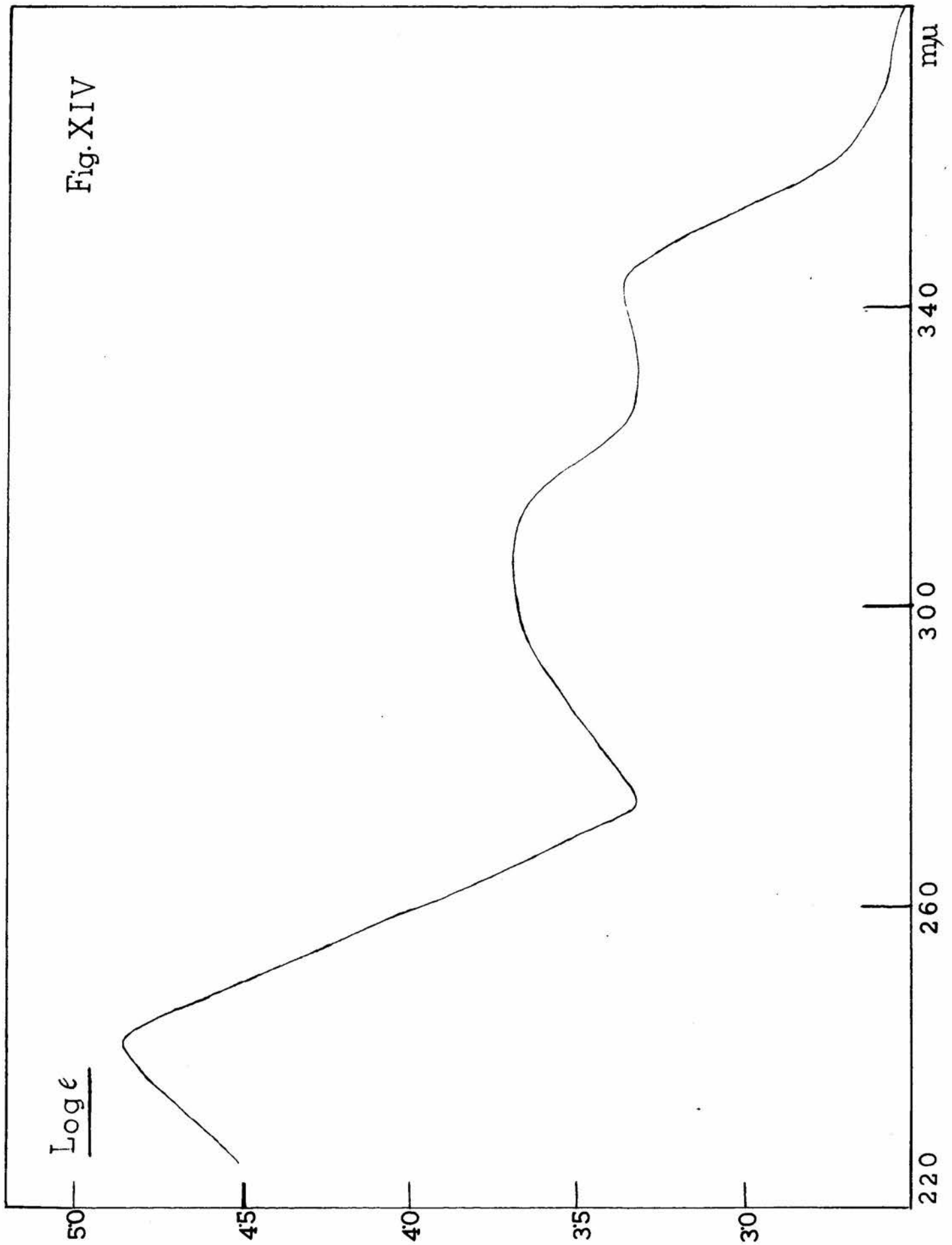


Fig. XIV
Log ε
5·0
4·5
4·0
3·5
3·0
220
260
300
340
mμ

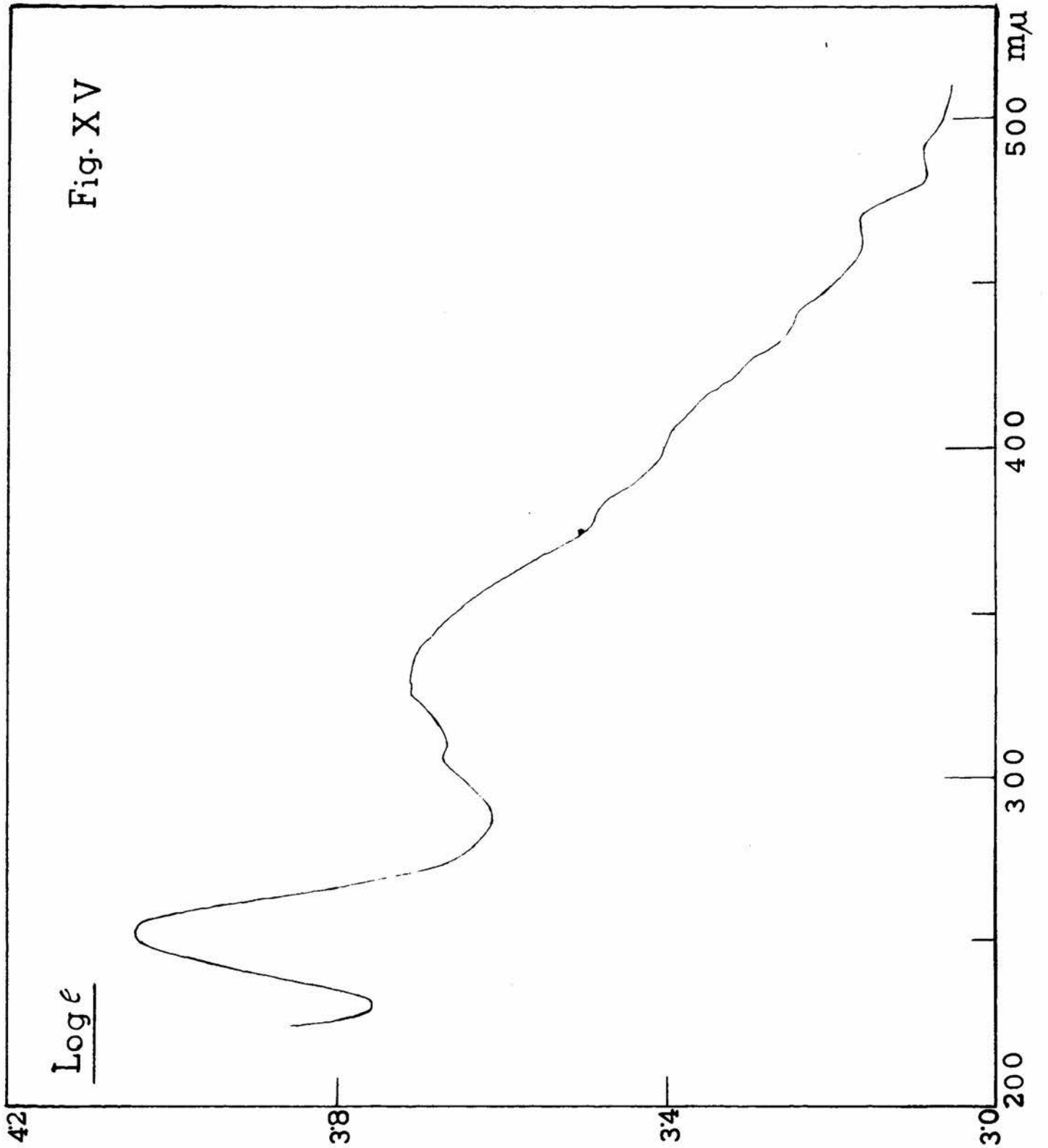


Fig. XV
Log ε
4·2
3·8
3·4
3·0
200
300
400
500
mμ

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PART II.

The Structure of Carminic Acid.

INTRODUCTION

The natural dyestuff cochineal was used as a dye and cultivated by artificial means in its native country, Mexico, at a remote period of history. It consists of dried bodies of the female insect Coccus cacti, which lives upon a species of cactus (the Nopalea coccinellifera S. -Dyck or Nopal), a plant which is found in the wild condition in Mexico and Central America. For the sake of the insect this plant is also cultivated in gardens which are termed Nopaleries. The insects are collected before the commencement of the rainy season and are killed by immersion in boiling paraffin or are enclosed in a linen bag and placed in an oven.

For a long time cochineal was an important mordant dye for wool and silk (red, crimson, scarlet, and violet shades) and found wide application in the dyeing industry. After 1830 the cultivation of cochineal was introduced also into Spain and Canary Islands, Algeria and Java; but with the development of the cheaper coal tar colours (the red azo dye-:stuffs), the consumption of cochineal as a dyestuff has gradually decreased, and at present time it is only employed in a minor degree. The commercially known carmin is prepared by extracting cochineal with water and precipitating the alu-:minium salt of the colouring matter (which still contains nitrogenous material) with alum. Carmin is used as a water-:colour, in rouge, to stain microscopic preparations and to

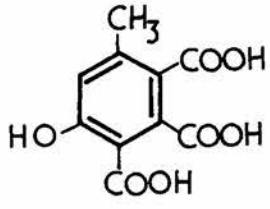
colour foodstuffs. Cochineal contains about 5% of the colour-:ing matter (Liebermann and Dimroth report 10% and 5% respect-:ively).

Carminic acid, the red pigment and the colouring prin-:ciple of cochineal, was first isolated by Pelletier and Caventou[1] in 1818 and was subsequently examined by numerous chemists. In 1858 Schutzenberger[2] succeeded in obtaining carminic acid in the crystalline condition by precipitating the colouring matter as its lead compound, decomposing this with hydrogen sulphide, and crystallising the liberated carminic acid from alcohol and ether. As carminic acid is very susceptible to aerial oxida-:tion its purification has been found rather troublesome by many workers. Miller and Röhde, [3] Schunk and Marchlewski,[4] and Dimroth[5] have described different methods for the purification of carminic acid.

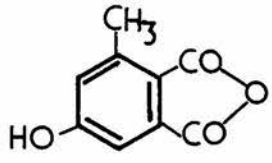
Carminic acid crystallises in small red prisms easily soluble in water and alcohol with a purple colour. It possesses no melting point, darkens at 130°, and at 250° becomes quite black. Of the many molecular formulae suggested for carminic acid by earlier workers, that advanced by Liebermann, Höring and Wiedermann[6] viz., $C_{22}H_{22}O_{13}$ was generally accepted as correct. In 1920 Dimroth modified this expression to $C_{22}H_{20}O_{13}$.

The structure of carminic acid was mostly clarified by the investigations of Liebermann, v. Miller, and notably Dimroth (1909-20.)

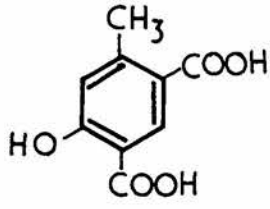
In 1897 Liebermann and Voswinckel[7] oxidised carminic acid with alkaline permanganate at the room temperature and obtained cochenillic acid (I) which, on heating, lost CO_2 and at 260° was converted into methyl hydroxyphthalic anhydride (II). They also found that cochenillic acid, on heating with water in a sealed tube at 170°, could be converted into α-coccinic acid which was later shown by them to be identical with m-hydroxy uvitic acid (III).[8]



I

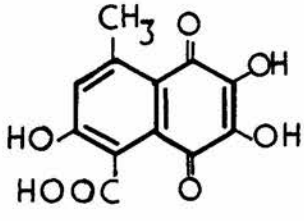


II

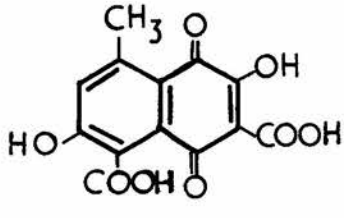


III

Later, Dimroth[9] oxidised carminic acid with acid permanganate at 0° and obtained carminazarin which he showed to possess the structure (IV). By oxidising carminic acid with hydrogen peroxide in the presence of catalyst,[5] Dimroth also succeeded in producing 2:6-dihydroxy-8-methyl-α-naphthoquinone-3:5-:dicarboxylic acid (V) which he oriented by comparing its colour reactions with those of synthetic 2:6-dihydroxy-α-naphthoquinone, and also by its conversion into carminazarin by treatment with acid permanganate.



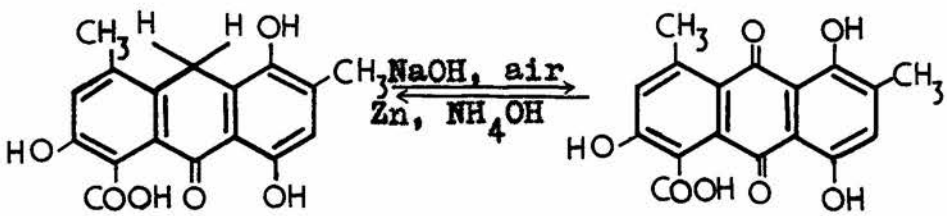
IV



V

Oxidative degradations to naphthoquinones, in this way, established a part of the nuclear structure of carminic acid.

The presence of an anthracene nucleus in carminic acid was revealed from the examination of its alkali fusion product, coccinin. This compound was first prepared by Hlasiwetz and Grabowski[10] as long ago as 1867, but nothing was known about its molecular structure until 1913, when Dimroth examined the compound in more detail and succeeded in assigning a structure to it. He found that an alkaline solution of coccinin is easily oxidised by air to coccinone which, on reduction with zinc dust and ammonia, is reconverted into coccinin. Dimroth[5] formulated these compounds as follows:

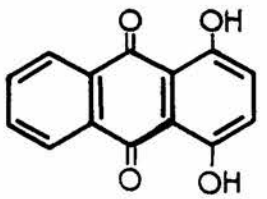


coccinin **coccinone**

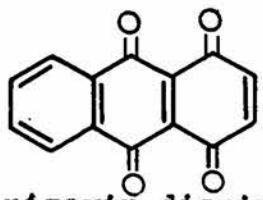
Thus coccinin was found to be related to its oxidation product coccinone in the same way that anthrone is related to anthra-:quinone, and this evidence pointed to an anthraquinonoid structure of carminic acid. It may be remarked that in agree-:ment with this carminic acid, on distillation with zinc dust, gave a small yield of anthracene and α-methyl anthracene.

On reduction with zinc and acetic acid,[11] carminic acid produced a leuco-compound, which was oxidised in alkaline

solution to a new pigment, desoxycarminic acid, which differed from carminic acid in containing one less oxygen atom. On oxidation desoxycarminic acid, like quinizarin, gave a di-:quinone which, by application of the Thiele reaction, was re-:converted into carminic acid just as quinizarin diquinone is converted into purpurin.[12]

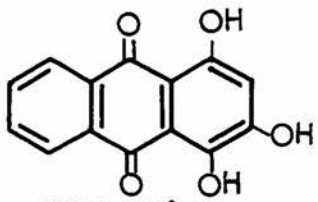


quinizarin

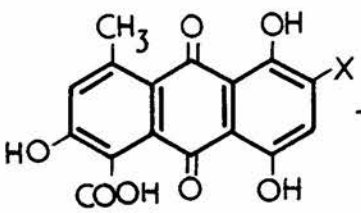


quinizarin diquinone

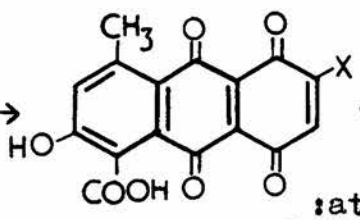
Thiele's acetylat-:ion and hydrolysis



purpurin

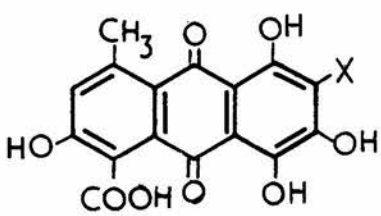


desoxycarminic acid



diquinone of desoxycarminic acid

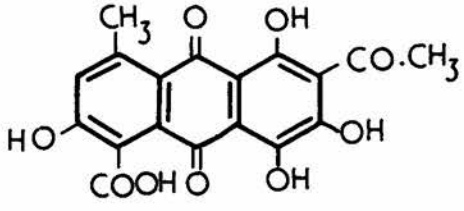
Thiele's acetyl-:ation and hydroly-:sis.



carminic acid

(X is a six carbon chain structure)

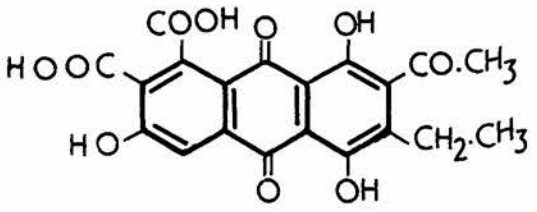
The location of the newly introduced hydroxyl group at the 3-rather than the 2- position was established from the ultra-violet and visible absorption spectrum of carminic acid, in analogy to known polyhydroxy anthraquinones, especially ker-:mesic acid (VI), the colouring matter of kermes. (Kermes[13] is also an insect dye and consists of the dried bodies of the insect coccus ilices.) It was used in ancient times as a scarlet mordant dye (Venetian scarlet); but afterwards it was displaced by cochineal. The pigment kermesic acid is a brick-red, water-insoluble substance. This compound was shown by degradative methods to possess structure (VI)[14] as long ago as 1915. The similarity of the structure of carminic acid with that of kermesic acid is noteworthy.



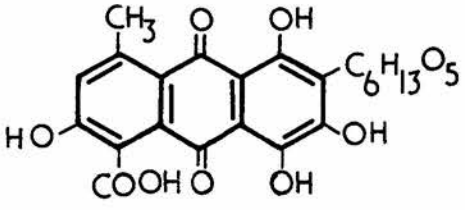
VI

It is also of interest to note that a third species of coccidoe insects, known as coccus lacca, gives another dye whose properties are comparable to cochineal. This is known as lac dye. Its deep red colouring principle, laccaic acid, is very similar in properties to carminic acid. Though the structure of laccaic acid[15] is not yet fully established, it is essenti-:ally a hydroxyanthraquinone carboxylic acid. The following

structure has been suggested:

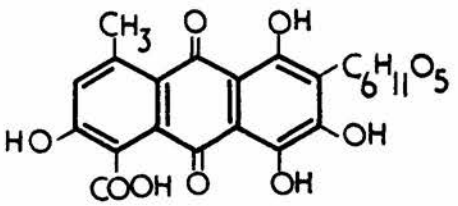


However, adding up all the facts described so far and on the basis of the empirical formula $C_{22}H_{22}O_{13}$, Dimroth, in 1913, formulated carminic acid:



It was previously observed by Miller and Röhde[3] that carminic acid, on being treated with acetic anhydride and sulphuric acid at the ordinary temperature, yielded on octa-:acetyl derivative. Since only four hydroxyl groups are present in the anthraquinone nucleus, it follows that the re-:maining four must be present in the side-chain, to which the composition $C_6H_{13}O_5$ has been assigned. This side-chain can only be a saturated univalent hydrocarbon radicle with five oxygen atoms, only four of which have been shown to be present as hydroxyl groups; hence it is concluded that the remaining oxygen atom is present in the ethereal, aldehydic, or ketonic

form, and its formula must then be modified[11] to $C_6H_{11}O_5$. Accord-:ingly, the empirical formula for carminic acid becomes $C_{22}H_{20}O_{13}$, and an examination of recorded analyses showed that they agree as well with this as with the older formula. Hence carminic acid is:

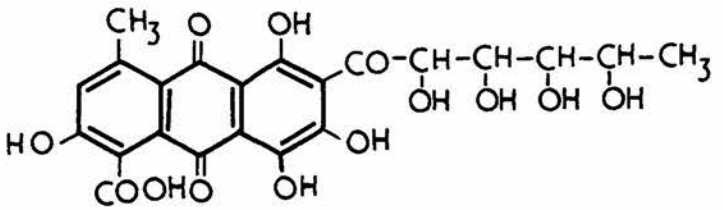


Carminic acid is optically active and has $[\alpha]_{645}^{15}$+ 51.6° in aqueous solution. This phenomenon cannot be attributed to the structure of the nucleus, since kermesic acid and also the less closely related laccaic acid, are optically inactive; it therefore appears that the side-chain contains at least one asymmetric carbon atom. Nothing more is known about the side-chain except that it is not bound to the anthraquinone as a glycoside, since carminic acid, on being refluxed with mineral acids, does not hydrolyse to give any sugar-like component.

The subject of the present study has been the elucid-:ation of the structure of this $C_6H_{11}O_5$ side-chain and the determination of its mode of linkage with the anthraquinone part.

It should be mentioned here that in 1926 the Japanese worker, I. Miyagawa, published a paper[16] on carminic acid where

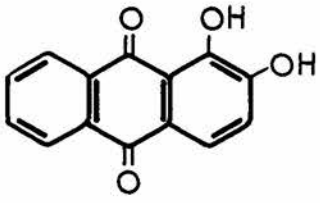
he suggested a structure for the side-chain. This is the only paper which describes an investigation on the side-chain of the carminic acid molecule. According to Miyagawa, carminic acid, when treated with ozone in aqueous solution, yields an optically active product from which, by oxidation, an optically active acid, $C_6H_{12}O_6$, is obtained. On reduction with sodium amalgam this acid yields a sugar, $C_6H_{12}O_5$, which is not identical with any of the known methylpentoses. By analogy with kermesic acid, Miyagawa suggested the following structure for carminic acid:



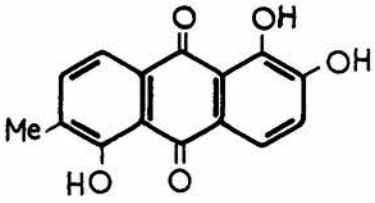
The evidence for this structure does not appear conclusive and such a structure is difficult to reconcile with the formation of coccinin when carminic acid is fused with alkali.

STRUCTURAL INVESTIGATION

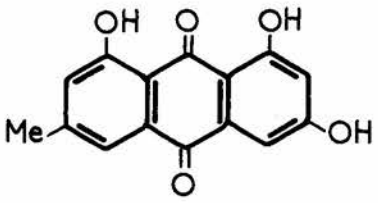
Glycosides of polyhydroxyanthraquinones are known to undergo ready hydrolysis like any other glycoside when heated with mineral acids. Examples are ruberythric acid[17] (giving alizarin, A, glucose and xylose), morindin[18] (giving morindone, B, and glucose) and frangulin[19] (giving emodin, C, and rhamnose) on treatment with dilute sulphuric acid.



A



B



C

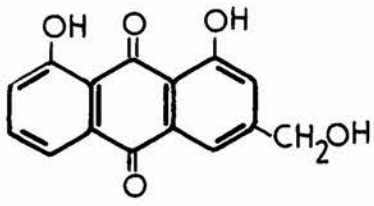
Carminic acid, which is structurally analogous to the above compounds, did not yield any sugar on heating at 100°C with N sulphuric acid for four hours or with 38% hydrobromic acid for six hours. Incubation of an aqueous solution of the colouring matter with the enzyme, emulsin, also did not afford any sugar. The failure to hydrolyse carminic acid with acid or an enzyme led to the conclusion that the side-chain, $C_6H_{11}O_5$, is not bound to the anthraquinone residue by an ordinary glycosidic link.

Two other compounds, in which the similar behaviour is encountered, have recently been studied in this department. One is barbaloin (occurring in the dried juice of the aloe plants) and the

other bergenin (found in roots and rhizomes of the plant, Bergenia crassifolia.) A brief account of these compounds is given here for clarification.

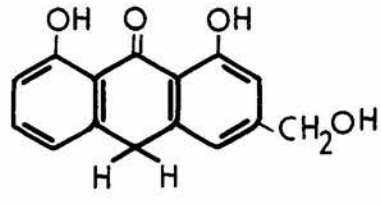
Barbaloin, $C_{21}H_{22}O_9$, is a lemon-yellow crystalline solid obtained by careful recrystallisation of commercial aloin of which it is the principal component. Aloin is commercially important as a constituent of many purgative medicines. Barba-:loin was first isolated over a hundred years ago and has been closely studied in attempts to determine its constitution.

On chromic acid oxidation, barbaloin was found to give a mixture[20] of aloe-emodin (VII) and rhein (VIII). Treatment with borax solution resulted in the breakdown of the molecule to aloe-emodin anthrone[21] (IX). These facts together with the formation of a hepta-acetate led earlier workers to the conclusion that barbaloin contained an anthrone or an anthranol nucleus combined with a sugar residue. This residue was not bound to the nucleus by a simple glycosidic link for it did not undergo hydrolysis under the conditions normally employed for hydrolytic fission of a glycoside.



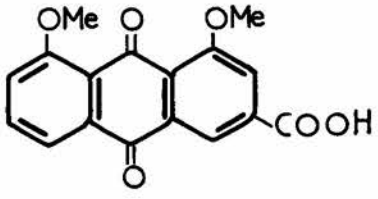
VII

VIII



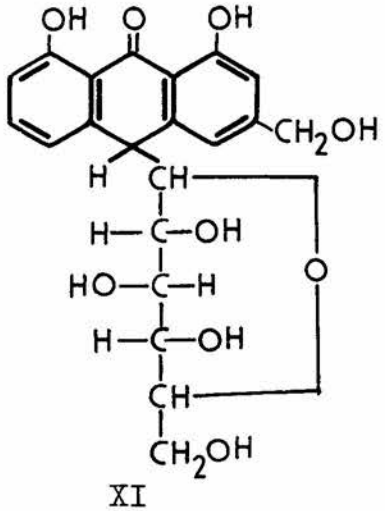
IX

Permanganate oxidation of the Purdie methylation product of barbaloin gave rhein dimethyl ether[22] (X) proving that the two phenolic groups must be free and not involved in linkage with the sugar residue.



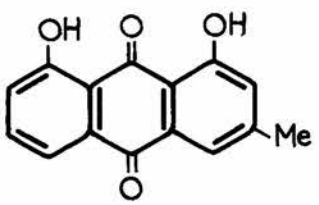
X

In 1952 Mühleman[23] succeeded in synthesising barbaloin. On condensing aloe-emodin anthrone with tetra-acetyl-α-D-gluco-:pyranosyl bromide in aqueous acetone containing sodium hydroxide, he obtained tetra-acetyl-barbaloin. Deacetylation yielded barba-:loin identical with the natural product. It would be expected that the synthetic product would be a glycoside derived from either the anthranol hydroxyl or the aliphatic hydroxyl group; but on the contrary Mühleman believed that the structure of his synthetic product (or barbaloin) is:



XI

Recently Birch and Donovan[24] have supported this structure by demonstrating that the ultra-violet absorption spectra were only consistent with an aloe-emodin anthrone rather than anthranol system in barbaloin and its derivatives. The sugar residue is not linked through the primary alcoholic hydroxyl group, as barbaloin, on hydrogenolysis in acetic acid with palladium charcoal, gave a deoxy-barbaloin[25] which formed a dimethyl ether with diazomethane (phenolic groups untouched). It differed from barbaloin only in having a methyl group in place of the hydroxymethyl at $C_{(3)}$, as oxidation gave what is almost certainly 3-methyl chrysazin:



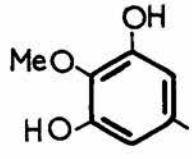
On oxidation with sodium metaperiodate,[25] barbaloin took up two mol. of reagent with the formation of one mol. of formic acid but no formaldehyde. This proved the presence of a vicinal triol system not terminating in CH_2OH, and was thus in harmony with the structure (XI).

Hay and Haynes[26] studied the infra-red spectra of barbaloin, its derivatives and other related compounds, and from this they also concluded that barbaloin contained an aloe-emodin anthrone

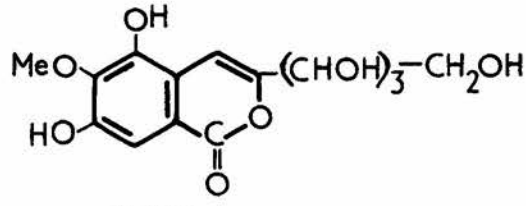
nucleus and the sugar residue is attached at the $C_{(10)}$. Oxidation of barbaloin with aqueous ferric chloride was shown by Cahn and Simonsen[22] to give aloe-emodin in good yield. By removing the inorganic materials from the mother-liquor of this reaction by ion-exchange technique, Hay and Haynes isolated a second product, D-arabinose.

Mühleman's structure for barbaloin is now accepted as correct. An unusual feature in this structure lies in the way in which the glucose and anthrone residue are united by a direct carbon-carbon linkage instead of a carbon-oxygen-carbon linkage. Another compound of this type (i.e., a C-glycoside) is bergenin.

Bergenin, $C_{14}H_{16}O_9$, crystallises in colourless prisms and is fairly soluble in water. Investigations by Tschitschibabin[27] made it clear that it consists of a 4-methoxy gallic acid nucleus (XII) linked with a polyhydroxy side-chain. The lactonic nature of the substance together with other facts led him to put forward the iso-coumarin type structure (XIII).



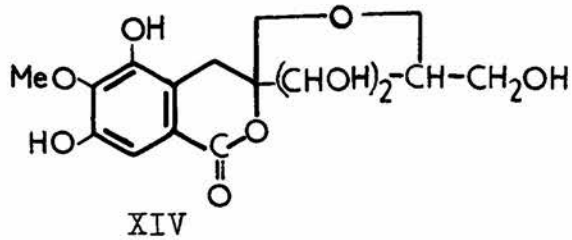
XII



XIII

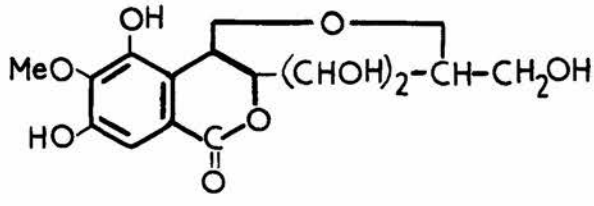
After about twenty years Shimôkorìyama[28] suggested the

structure (XIV):



XIV

Recently Hay and Haynes (1957) have proved that the structures (XIII and XIV) are incorrect. According to them bergenin is:



The underlying evidence* in support of this structure is the following:-

(1) On oxidation with sodium metaperiodate, the dimethyl ether of bergenin (XV), prepared by the action of diazo-:methane on bergenin) consumed one mol. of the reagent. The same compound, after the lactone ring had been opened, took up the two mol. of periodate.

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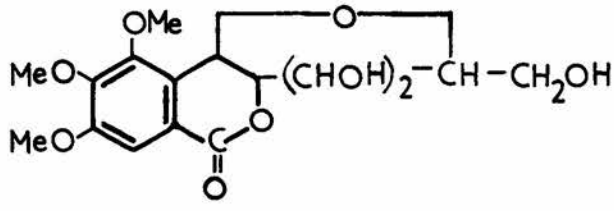
* Private communication.

(2) Condensation of tetra-acetobromo glucose with the methyl ester of 4-methoxy gallic acid, in presence of sodium methoxide, followed by hydrolysis and consequent lactonisation gave bergenin, identical with the natural product.



XV

It is reported by Dr. Hay that bergenin (containing lactone ring), on periodate oxidation at 0°, takes up 2.1 mol. of the reagent. This is due to the fact that the phenolic groups are also oxidised.

Another important observation is that ring-opened bergenin, unlike barbaloin, does not yield any sugar on oxidation with ferric chloride. This behaviour can, probably, be attributed to the aromatic nature of the nucleus to which the sugar residue is attached by a C-C linkage.

As it seemed from its behaviour towards hydrolysis that carminic acid is not an ordinary glycoside, it was conceived that it might be a C-glycoside like barbaloin and bergenin. To know whether this is really the case, the reactions of carminic acid

were studied in parallel with those of barbaloin.

Carminic acid was oxidised with ferric chloride under the conditions that were employed in the case of barbaloin by Hay and Haynes.[26] During the process no detectable amount of solid separated from the solution (cf. separation of aloe-emodin from barbaloin). The solution, which contained the oxidation products, was freed from inorganic materials by ion exchange technique and examined by paper chromatography for the presence of sugars. At first no sugar could be detected. Only when the solution was reduced to a very small bulk (i.e. the volume of 2-3 drops when 500 mg. of carminic acid was oxidised) the concentration of sugar was just enough to be detected by paper chromatography. In this case the paper chromatogram, after being sprayed with aniline oxalate, disclosed two sugar spots, one intensely pink in colour and the other yellow. The latter spot fluoresced greenish yellow in the ultra-violet light. Further chromatographic examinations indicated that the pink spot was due to arabinose and the yellow one was probably due to glucose. A paper chromatogram, in which the sugars produced by oxidation of carminic acid were allowed to run along with glucose, xylose and arabinose, is described below:

TABLE I.

Solvent:n-Butanol (10)/Pyridine (3)/Water (3) - 46 hr. run at 22°.

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	10.9
Arabinose	0.80	8.8
Glucose	0.71	7.8
From Carminic acid	0.79 (pink)	8.7
	0.66 (yellow)	7.2

Barbaloin was next oxidised with ferric chloride. The separated solid (aloe-emodin) was removed and the mother-liquor was freed from inorganic materials by a conventional ion exchange technique. The sugar solution thus obtained was chromatographed on paper together with the sugar solution obtained from carminic acid.

The solution from barbaloin yielded two spots (one pink due to arabinose and the other yellow). The distances to which they moved were equal to those of similar spots obtained from the carminic acid degradation solution.

This was confirmed by chromatograms run in two different solvent systems. (Tables II and III).

TABLE II

Solvent: n-Butanol (10)/Pyridine (3)/Water (3).
- 27 hr. run at 25°.

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	6.6
Arabinose	0.82	5.4
Glucose	0.69	4.6
From Barbaloin	0.80(pink) *	5.3
	0.68(yellow) *	4.5
From Carminic acid	0.82(pink)	5.4
	0.69(yellow)	4.6

* The yellow spots showed greenish yellow fluorescence (similar to glucose spots) in ultra-violet light.

TABLE III

Solvent: n-Butanol (2)/Acetic acid (1)/Water (1)
- 22 hr. run at 21°

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	5.35
Arabinose	0.82	4.4
Glucose	0.50	2.7
From Barbaloin	0.82(pink)*	4.4
	0.52(yellow)*	2.8
From Carminic acid	0.83(pink)*	4.45
	0.54(yellow)*	2.9

* The yellow spots showed greenish yellow fluorescence (similar to glucose spots) in ultra-violet light.

This comparative study disclosed the following facts:-

(1) Like barbaloin, carminic acid produces arabinose on oxidation with ferric chloride although the yield is extremely poor (cf. Bergenin)

(2) In addition to arabinose, both carminic acid and barbaloin probably give glucose. The yield in this case also is very small.

(3) In carminic acid the sugar side-chain seems to be glucose which is attached to the anthraquinone residue by a C-C linkage as in barbaloin.

Ozonolysis of carminic acid and barbaloin gave similar

results. The two substances were ozonised under identical conditions and the ozonides were decomposed by steam-distillation. The distillates, in both cases, were found to contain formaldehyde (detected by the colour reaction with chromotropic acid) and the residues, after extracting with ether, were treated with lead acetate whereby most of the impurities were precipitated and almost pure sugar solutions were obtained. Sugar solutions from carminic acid and barbaloin were chromatographed on the same paper for comparison. In each case a pink and a yellow spot appeared on the chromatogram and the rates of movement of the same-coloured spots were equal. This is evident from the data recorded in the following Tables:-

TABLE IV

Solvent: n-Butanol (10)/Pyridine (3)/Water (3)
- 22 hr. run at 25°

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	5.7
From Barbaloin	0.82(pink)	4.7
	0.65(yellow)	3.75
From Carminic acid	0.80(pink)	4.6
	0.64(yellow)	3.7

TABLE V

Solvent: Ethyl acetate (3)/Acetic acid (3)/Water (1)
- 22 hr. run at 25°

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	15.6
From Barbaloin	0.90(pink)	14.1
	0.81(yellow)	12.75
From Carminic acid	0.89(pink)	13.9
	0.81(yellow)	12.7

Paper chromatography with spots of different sugars as markers indicated that the pink and the yellow spots were due to arabinose and glucose respectively:

TABLE VI

Solvent: n-Butanol (10)/Pyridine (3)/Water (3)
- 40 hr. run at 24°

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	8.5
Arabinose	0.82	7.05
Glucose	0.70	5.95
From Barbaloin	0.82(pink)*	7.05
	0.70(yellow)*	5.95
From Carminic acid	0.81(pink)*	6.9
	0.69(yellow)*	5.85

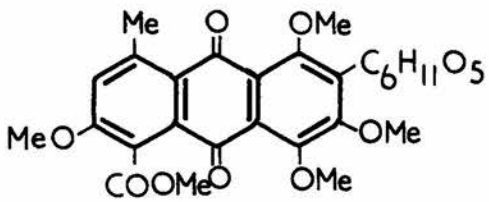
* Like the glucose spot, the yellow spots showed greenish yellow fluorescence in the ultra-violet light.

Ozonolysis of carminic acid was found to produce a higher yield of sugars (310 mg. of the crude syrup from 3 g. of carminic acid) than the ferric chloride method. Arabinose and glucose were separated and purified by chromatography (thrice) of the crude syrup on thick filter papers, cutting the papers at the proper position and eluting with water. Arabinose was characterised by making its benzoyl hydrazone derivative (m.p. 184-187°, decomp.; lit. m.p. 184-188°). Mixed melting point with an authentic specimen (benzoyl hydrazone of D-arabinose) was 182-186° (decomp.). Determination of the optical rotation was not possible due to the small quantity of the derivative obtained.

The glucose solution was treated with a solution of p-nitroaniline in methanol containing a little HCl, but p-nitro-anilide derivative could not be obtained presumably due to the small quantity of sugar present. The similar behaviour of carminic acid and barbaloin in the ozonolysis reaction (as evidenced from the paper chromatography and the isolation of D-arabinose) suggested that the former is a C-glucoside like the latter.

As already mentioned, barbaloin takes up two mol. of sodium metaperiodate. It was expected that carminic acid would show a similar result. But, on the contrary, it consumed six mol. of the reagent when oxidised at 0°. This was considered to be due to the oxidation of the more susceptible (compared to barbaloin) phenolic hydroxyl groups (cf. Bergenin). In order to avoid this, the phenolic hydroxyl groups of carminic acid were protected by

selective methylation with diazomethane producing a yellow, difficultly crystalllisable solid (microscopic needles, m.p. 185-188° with decomp.). Analysis proved that the substance was methyl tetra-O-methyl carminate (XVI). (Found: C, 57.0; H, 5.4; OMe, 27.9% [5.04 groups], 28.1% [5.10 groups]. Calc. for $C_{22}H_{15}O_8(OMe)_5$: C, 57.7; H, 5.3; OMe, 27.6%).

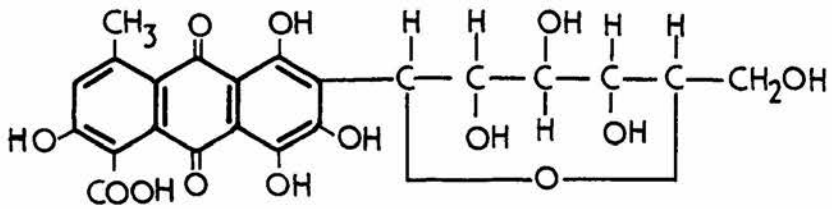


XVI

Methyl tetra-O-methyl carminate, on oxidation with sodium metaperiodate, was found to take up two mol. of the reagent (thus confirming that the phenolic groups were responsible for the extremely high results in the unmethylated compound). This is in agreement with the fact that the $C_6H_{11}O_5$ is a hexose —— and by analogy with the others a glucose residue. It must be pointed out that one of the reasons which discount Miyagawa's structure is provided in this experiment. If carminic acid had the structure proposed by him, it should have consumed three mol. of the periodate instead of two.

After the oxidation of methyl tetra-O-methyl carminate with sodium metaperiodate was complete, the reaction mixture was steam-

distilled. The distillate was found to contain formic acid (detected[29] by its reducing action on mercuric chloride). When barbaloin was treated similarly, formic acid was also detected in the distillate. The formation of formic acid by periodate oxid-:ation proved that carminic acid contained the system -CHOH-CHOH-CHOH-. The glucose side-chain, therefore, must be present in the pyranose form (and not furanose) as in barbaloin. Hence carminic acid is 1:3:4:6-tetrahydroxy-8-methyl-2-glucopyranosyl-anthra-:quinone-5-carboxylic acid:



It is apparent that the formation of coccinin, on caustic potash fusion of carminic acid, is not impossible from the view point of this structure. In order to verify this an experiment was made in which glucopyranosyl benzene was fused with caustic potash and the mixture distilled. The distillate (colourless liquid) was subjected to gas chromatography; but no toluene could be detected. However benzene and hydroxyanthraquinones are so different that a negative result is not against the proposed structure.

The infra-red spectrum of carminic acid showed complex absorptions in the carbonyl region and therefore did not prove very helpful in correlation. This was due to the many possibilities which existed for the hydrogen-bonding of the three carbonyl groups. The compound (in nujol) showed strong absorptions at 1708, 1693, 1677 and 1606 $cm.^{-1}$. Bands of medium intensity at 1648 and 1632 $cm.^{-1}$ were also present.

EXPERIMENTAL

Carminic acid required for this investigation was at first obtained from B.D.H. but the material (labelled 'purified') proved (as will be described below) unsuitable for the purpose as it contained only 5% of water-soluble substance. Examination of the aqueous solution by paper chromatography revealed the presence of two other substances besides carminic acid. These were probably the air-oxidised products. It was experienced that when carminic acid (pure) was exposed to air, it quickly changed into a purple coloured water-insoluble solid which gradually turned into a black sticky mass. This great susceptibility of carminic acid towards aerial oxidation does not seem unlikely when one considers the presence of three hydroxyl groups in the same arom-:atic ring (Hydroxyhydroquinone system). The cause of the B.D.H. purified sample deteriorating was apparently this sort of oxida-:tion. Attempts were made to purify it but without success. It was accordingly decided to isolate carminic acid from the insects (coccus). (The methods that are available for this have already been referred). Coccus (supplied by T. & H. Smith Ltd., Edinburgh) was extracted with ethanol in a soxhlet and the extract gave an impure oil which could not be further purified to yield crystalline carminic acid (the purification method of Miller and Rohde[3] did not work). However, pure crystalline carminic acid was isolated

from insects through a different course which is actually a combination of Schutzenberger's and Dimroth's methods. The detailed procedure is described in this section. It is important to note that all processes in the isolation of carminic acid should be carried out in an inert atmosphere. The pure material should also be stored in absence of oxygen.

Attempted purification of B.D.H. Carminic Acid.

B.D.H. carminic acid (10 g.) was continuously extracted with ethanol (400 ml.) in an atmosphere of carbon dioxide. After six hours' extraction the cooled extract was filtered and concentrated again in absence of air. On cooling, impure carminic acid separated from the solution as small black granular particles which were collected by filtration and the mother-liquor was then further concentrated. After some time, a small amount of solid separated which was again filtered off and dried in vacuum over silica gel. This solid (220 mg.) looked bright red and seemed to be much purer than the starting material. Since paper chromatography indicated that the substance still contained impurities, it was dissolved in ethanol and the solution was chromatographed on magnesium sulphate (dried at 200°C). Elution with ethanol

caused the mixture to pass through the column without separation. Chromatography on keiselguhr gave the same result. The filtrate from the column was evaporated to dryness but the residue could not be crystallised from methanol, ethanol or isoproply alcohol.

The mother-liquor obtained from the extract, on further concentration, turned into a thick oil which did not crystallise.

Attempt was made to purify B.D.H. carminic acid by the method of Miller and Röhde[3]. But this was not successful.

Attempted preparation of Carminic acid from insects (by Soxhlet extraction)

The dried insects (400 g. coccus) were thoroughly powdered and then extracted in Soxhlet for five hours with ligroin in order to remove the wax adhering to the insects. The insect powder was dried and extracted with ethanol for seven to eight hours. The extract was filtered, concentrated and after twelve hours impure carminic acid separated from the solution as a dark coloured solid which could not be further purified. Removal of solvent from the mother-liquor gave a dark oil which also did not yield any crystals.

Preparation of Carminic acid from insects (by lead salt method; cf. Schunk and Marchlewski;[4] Dimroth and Scheurer[14]).

The dried insects (250 g., coccus) were thoroughly powdered and suspended in hot water (8 litres at 60-65°C). The mixture was stirred for an hour and then was kept at room temperature overnight. The aqueous extract was filtered through glass wool and

then twice by suction through double filter papers. The latter process was very slow due to the viscous nature of the extracts.* Finally the filtrate was once again filtered by suction.

On adding lead acetate solution to the clear filtrate, lead carminate precipitated as a blue solid which gradually settled at the bottom of the flask. Lead acetate solution was added until the precipitation was complete. The clear colourless supernatant liquid was decanted from the precipitate which was then washed with a large volume of water with stirring and decantation. The aqueous suspension was centrifuged and the solid centrifugate was thoroughly washed (twice) with absolute ethanol. An ethanol suspension was again centrifuged and the ethanol removed. The lead salt was transferred to a tall cylinder (16" x 2¼") and was treated in suspension in one litre of absolute ethanol with H_2S gas for eight hours with stirring. The lead sulphide was allowed to sediment and the ethanolic solution of carminic acid was decanted. The precipitate was treated with H_2S for 2-3 hours as above. The combined carminic acid solutions were filtered** and the clear red filtrate was evaporated to dryness under reduced pressure in nitrogen (freed from moisture and

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* If celite is used as an aid in filtration then, on adding lead acetate solution to the filtrate, lead carminate is not precipitated but remains in the colloidal state which causes much difficulty in purification and also a low yield of carminic acid.

** The precipitated lead sulphide (which adsorbed some carminic acid) was extracted in soxhlet with ethanol. Although a fair amount of carminic acid was present in the extract, it could not be obtained pure.

oxygen). The residue was dissolved in ca. 120 ml. of methanol and the solution, on standing overnight in nitrogen, yielded beautiful bright-red crystals (microscopic prisms) of carminic acid. The crystals were collected and washed with a small volume of ice-cooled methanol and finally they were dried in vacuo over silica gel. The carminic acid thus obtained was shown to be chromatographically pure. (R_f 0.17 in n-propanol (6)/Conc. ammonia (3)/water (1); 0.12 in n-butanol (3)/pyridine (1)/water (1). The dried crystals were stored in a dark coloured bottle which was filled with nitrogen and tightly stoppered. The mother-liquor, on further concentration, gave another crop of crystals. Total yield of carminic acid was 12.1 gm. (ca. 5%). Carminic acid did not melt but darkened at 120°, and at 180° turned completely black. Found: C, 52.98; H, 4.40; Calc. for $C_{22}H_{20}O_{13}$: C, 53.65; H, 4.07.
Infra-red (Nujol): 1708 s, 1693 s, 1677 m, 1648 m, 1632 m, 1606 s, 1566 s, and 1509 cm.$^{-1}$.

Attempted acid hydrolysis - Carminic acid was heated at 100° with N sulphuric acid for four hours or with hydrobromic acid (38%) for six hours. The solutions were examined by paper chromato-:graphy, but no sugars could be detected.

Attempted enzymic hydrolysis - A solution of carminic acid (50 mg.) in water (5 ml.) was incubated at 37° with an aqueous solution (5 ml.) of emulsin (50 mg.) containing acetate buffer

(p_H 5, 1 ml.). Aliquots were removed at intervals and examined by paper chromatography for the presence of sugar. After four days' incubation no sugar had been detected.

Periodate oxidation - An aqueous solution of carminic acid (14 mg.) and a 0.1 M solution of sodium metaperiodate (4 ml.) were mixed. The mixture was allowed to stand at 0° in the dark for four hours. On titration, the periodate uptake was found to be equivalent to the consumption of 6.2 moles of periodate per mole of carminic acid.

Preparation of methyl tetra-O-methyl carminate.- Carminic acid (2 g.) was dissolved in warm anhydrous methanol (150 ml.). The solution was cooled to ca. 5° and diazomethane (ca. 2.5 g.) in ether (100 ml.) slowly added. Vigorous evolution of nitrogen took place and some carminic acid precipitated. The mixture was allowed to stand at room temperature. After 16 hours the colour of the solution was pale brownish red and most of the precipitated carminic acid had redissolved. The solution was warmed on a water bath (60-65°) to remove excess diazomethane and ether. The resulting methanolic solution was cooled, filtered, and the filtrate distilled under reduced pressure in an atmosphere of nitrogen. On complete removal of the solvent, a very viscous dark brownish red oil was obtained. The oil was found to be very soluble in alcohol, benzene, chloroform, acetone, dioxane, ethyl acetate, acetic acid , water etc. and completely insoluble

in ether and ligroin. Attempts were made to crystallise the methylated product from single solvents or solvent pairs (ethanol-ligroin, methanol-ligroin, ethanol-ether, benzene-ligroin, benzene-chloroform, ethylacetate-ligroin, chloroform-dioxane-ether) using methods of cooling at room temperature or at a lower temperature with dry ice/acetone and slow evaporation, but all were unsuccessful. Purification of the oil by adsorption chromatography was then attempted.

The oil was dissolved in benzene and put on a column of alumina. Elution with benzene, chloroform-benzene, chloroform, ethanol-benzene proved unsatisfactory. Chromatography on magnesium carbonate (chloroform) or keiselguhr (benzene) also did not give good results. However magnesium carbonate as adsorbent with benzene as the developing liquid was found to be fairly satisfactory.

A solution of the oil in benzene was put through a column (12 x 2.1 cm.) of magnesium carbonate and elution was continued with benzene. Carminic acid and other impurities remained adsorbed at the top of the column as a broad red band. The product separated as a brownish yellow band which, on elution, gave a deep yellow solution. This solution was concentrated (ca. 10 ml.) and again chromatographed as before. The bright yellow eluate was concentrated giving a solution which looked brownish red by reflected light and yellow by transmitted light. Since no crystalline material had separated from this solution

after 16 hours, the solvent was removed *in vacuo* under nitrogen leaving a yellow glass, which had the same solubility characteristics as the crude product and crystallisation was found to be difficult (cf. carminic acid). However, on slow evaporation of a solution of the substance in a mixture of benzene and ligroin some yellow solid separated after a period of two weeks. This was filtered, quickly washed with cold benzene two to three times and dried in a vacuum dessiccator over paraffin wax. The dried solid (yellow microscopic needles) melted between 185-188° (decomp.).

Analysis

Found: C, 57.00; H, 5.41; OMe, 27.85% (5.04 groups), 28.14% (5.10 groups). Calc. for $C_{22}H_{15}O_8(OMe)_5$: C, 57.65; H, 5.34; OMe, 27.58%.

As a result of a number of experiments, it was found that the optimum conditions required for the methylation of carminic acid necessitate the use of -

(a) absolutely dry methanol[30]
(b) absolutely dry ethereal diazomethane[31]
(c) a concentrated solution of carminic acid in methanol.

In one reaction a solution of carminic acid (2 g.) in methanol (450 ml.) was treated with diazomethane (*ca.* 5 g.) in ether (200 ml.) and the mixture set aside at room temperature. Excess methanol was used to avoid precipitation ofcarminic acid. After two to three days the intense red colour of the solution

still persisted indicating that the reaction was far from complete. The ether was removed and the residue treated with the same amount of ethereal diazomethane as before. The red colour of the solution still persisted, even after a week. After removal of ether, the methanolic solution was concentrated to ca. 150 ml. and again treated with the same amount of ethereal diazomethane. This time the solution, on standing overnight, became brownish yellow which indicated complete methylation of carminic acid.

Periodate oxidation of methyl tetra-O-methyl carminate

An aqueous solution of methyl tetra-O-methyl carminate (25.1 mg.) and a .05 M solution of sodium metaperiodate (10 ml.) were mixed, the volume made up to 20 ml. with distilled water and the solution allowed to stand at 0°. The progress of the oxidation was followed by the titration of aliquots (2 ml.). The reaction was complete in two hours and the periodate uptake was equivalent to the consumption of 2.1 moles of periodate per mole of the tetramethyl ether.

It was noticed that the clear, yellow solution of methyl tetra-O-methyl carminate became turbid within one hour from the time of addition of periodate solution, the separation of water-insoluble solid being due to the destruction of the sugar hydroxyl groups.

In another reaction, when the oxidation was complete, the solution was steam-distilled. Formic acid was detected in the distillate by the following method:

To a few drops of the distillate (acidic) in a small porcelain crucible were added one drop of mercuric chloride solution (10%) and one drop of buffer solution (1 ml. glacial acetic acid and 1 g. sodium acetate per 100 ml. water). The mixture was taken to dryness in an air oven and the residue treated with a drop of dilute ammonia. An intense black colour appeared.

An aqueous solution of barbaloin was also oxidised with sodium metaperiodate. When the reaction was complete (three hours) the solution was steam-distilled. The presence of formic acid in the distillate was detected by the above method.

Ferric chloride oxidation of Carminic acid

A solution of carminic acid (1 g.) and ferric chloride (5 g.) in water (20 ml.) was heated under gentle reflux at 105-110° for four hours. The dark reddish-violet solution was cooled and filtered. The filtrate was divided into two equal portions which were purified in two different ways in order to isolate any sugar formed.

(a) One portion of the solution was passed through columns of Amberlite resin I R - 120 (H) until the eluate was free from ferrous ions. The solution thus obtained was passed through columns of Amberlite resin I R - 4B (OH)* until all chloride ions had been removed. The neutral solution was still brown coloured and could not be decolourised on passing it again through columns

* Amberlite resin IR-4B(OH) should be well washed with dil. HCl and regenerated with NaOH before use, otherwise efferves-:cence takes place on passing the solution through the column.

of cation and anion exchange resins. The brown solution was, therefore, concentrated* to ca. 10 ml. and examined by paper chromatography for the presence of sugar, the developed paper chromatograms being sprayed with aniline oxalate and heated. No sugar could be detected. The solution was then concentrated to 5 ml., 2 ml., and 1 ml. and chromatographed on paper and in no case was any sugar detected although in the last case (i.e. when the volume of the solution was 1 ml.) a very faint spot was observed. The solution was therefore concentrated to a volume of two to three drops and again chromatographed. This time, after spraying the dried paper with aniline oxalate and heating, two intensely coloured spots (one pink and one yellow) appeared. Table I (page166) gives the rate of movement of these spots compared with those of xylose, arabinose and glucose. It was concluded, therefore, that carminic acid, on oxidation with ferric chloride, gave arabinose and probably also glucose but the yield of the sugars was very poor.

(b) In another portion of the solution iron was removed by precipitation with ammonia and the concentrated filtrate (ca. 5 ml.), was passed once through a column of Amberlite resin IR-120(H) and then through several columns of Amberlite resin IR-4B(OH) until the eluate was completely free from chloride ions.** The eluate was colourless and on concentrating to a

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* Concentrations were carried out by distillation under reduced pressure unless otherwise stated.

** Even a very small amount of chloride ion in the solution will greatly affect the paper chromatography, because a highly concentrated solution is needed for this purpose.

small volume (ca. 2 ml.) became pale yellow. This was further concentrated to ca. 0.5 ml. and the solution spotted on paper and chromatographed along with spots of glucose, xylose and arabinose. The result is given below:

TABLE VII

Solvent: n-Butanol (10)/Pyridine (3)/Water (3)
- 17 hr. run at 25°C

Sugar	R_{xylose}	Inches from starting line
Xylose	1.0	5.3
Arabinose	0.83	4.4
Glucose	0.69	3.7
From Carminic acid	0.83 (pink)	4.4
	0.70 (yellow)	3.75

Ferric chloride oxidation of Barbaloin

cf. Cahn and Simonsen,[22]; Hay and Haynes[26])

A solution of barbaloin (1 g.) and ferric chloride (5 g.) in water (20 ml.) was heated under gentle reflux at 105-115° for four hours. A dark-brown solid separated from the hot solution. The solution was cooled and filtered, the residue rejected and the dark red filtrate extracted thrice with amyl alcohol. The pale yellow aqueous layer was separated and passed through columns of Amberlite resin IR-120 (H) until the eluate was free from ferrous ions. The colourless solution thus obtained was passed through columns of Amberlite resin IR-4B (OH)

until all chloride ions had been removed. The neutral solution was concentrated to a small volume (ca.10 ml.) and spotted on paper. The solution (purified by NH_4OH method (b)) obtained by oxidising carminic acid with ferric chloride was also spotted on the same paper along with xylose, arabinose and glucose. The papers were chromatographed in two different solvent systems which gave the same results described in Tables II and III (p.167 and 168).

Ozonolysis of Carminic Acid

Carminic acid (3 g.) was dissolved in distilled water (60 ml.) by slightly warming on the water bath and the solution, after cooling, was transferred in a Drechsel bottle (150 ml.). The bottle was kept fully immersed in cold water and a stream of ozonised oxygen (2 per cent ozone) from a high-tension discharge apparatus (75,000 volts) was passed through the solution until its colour changed from red to brownish yellow (ca. 3 hr.). The ozonised product was then steam distilled and the distillate (ca. 300 ml.) was collected. The distillate gave a positive test for formaldehyde with chromotropic acid. The residue, which turned dark brown during distillation, was extracted thrice by shaking with ether (3 x 200 ml.) in a separating funnel. The aqueous phase removed and evaporated to dryness under reduced pressure when a dark brown syrup was obtained which smelled like caramel.

The syrup was dissolved in water (15-20 ml.) and the solution

(A) was used for paper chromatography. Three spots of this solution were put on a paper strip and were allowed to run by upward displacement in ethylacetate (3)/acetic acid (1)/water (3) - top phase. The air dried paper was photographed in ultra-violet light, the photograph showing that chromatographic separ-:ation of solution (A) in this solvent system resulted in the separation of five components which absorbed ultra-violet light (R_f 0.90, 0.70, 0.65, 0.51 and 0.36). The paper chromatogram was then cut into three thin strips each of which contained the chromatogram of one original spot. The paper strips were sprayed with different reagents and the results are given in the following Table:-

TABLE VIII

Distance of the solvent front from the starting line = 10.8 inches.

Paper Strip No.	Spraying reagent	Number and colour of spots	Inches* from the starting line	Inference
1	$KMnO_4$/ KIO_4	one long yellow streak	3.6 to 5.5	Sugar-like material present[32]
2	Aniline oxalate	one pink spot	4.6	reducing sugar present, probably pen-:tose.
3	KI/KIO_3/ Starch	two blue spots	(i) 4.8 (ii) 9.6	Acids present[33]

* As the solution (A) was very impure, R_f values have little significance.

The solution (A) was then purified in the following way:

The solution was diluted with water (ca. 200 ml.) and a saturated aqueous solution of lead acetate added to it when a yellowish-grey solid precipitated. When the precipitation was complete,,the solid was removed by filtration under suction. The filtrate was slightly yellow in colour. In order to remove the excess lead acetate, H_2S gas was passed through the filtrate. The mixture was then warmed on a water bath and PbS was filtered off. The colourless filtrate was concentrated to a small volume (ca. 20 ml.) and again H_2S gas was passed through it when some more PbS precipitated. This was removed by filtration and the filtrate was completely evaporated to dryness under reduced pressure. The residue thus obtained was a pale yellow syrup which was dissolved in water (5 ml.) and the solution (B) was used in paper chromato-:graphy.

Ozonolysis of barbaloin (cf. ozonolysis of carminic acid)

Ozonised oxygen (2 per cent ozone) was passed through an aqueous solution (50 ml.) of barbaloin (200 mg.) for about 5 hours. After first few minutes the colour of the solution changed from yellow to brown and finally it was yellow again. During oxonolysis the reaction vessel was kept immersed in cold water. The reaction product was then steam distilled and the distillate (ca. 300 ml.) was collected. The distillate gave a positive test for formaldehyde with chromotropic acid. The residue, which

turned dark brown during distillation, was extracted thrice by shaking with ether (3 x 200 ml.). The dark brown aqueous layer was separated and treated with a saturated aqueous solution of lead acetate. A dirty yellow solid precipitated. When the precipitation was complete the solid was removed by filtration under suction. The solution was repeatedly filtered until the filtrate was absolutely clear. H_2S gas was then passed through the filtrate, after which it was warmed on the water bath for few minutes and the precipitated PbS was filtered off. The colourless filtrate was concentrated to a small volume (ca. 5 ml.) and again H_2S gas was passed through it when some more PbS pre-:cipitated. This was filtered off and the colourless filtrate was evaporated to complete dryness under reduced pressure. An almost colourless syrup was obtained. The syrup was dissolved in water (2 ml.) and the solution (C) was used for paper chromato-:graphy.

Paper chromatography

The spots of the solutions (B) and (C), obtained from the ozonolysis reaction of carminic acid and barbaloin respectively were placed on the same paper and were allowed to run in parallel. Aniline oxalate was used as the spraying reagent. Two different solvent systems were examined. Descriptions of the chromatograms have been given in Tables IV and V (p.169-70). It is clear from the above figures that carminic acid as well as barbaloin produce the same sugars upon ozonolysis. The pink spots in both cases

should be due to arabinose and the yellow spots may be due to glucose. A paper was therefore run with spots of solutions (B) and (C) along with xylose, arabinose and glucose as markers. The result has already been given in Table VI (p.170).

Separation and purification of sugars

Strips of thick filter papers (Whatman No. 3 mm) were continuously extracted with benzene-ethanol mixture (1:1) for a few hours and then dried. Sugar solution (B), obtained from the ozonolysis reaction of carminic acid, was concentrated to a small volume (ca. 2 ml.) and the resulting syrup was placed in the form of several small drops (2-3 mm. diam.) along a line drawn on each paper strip. The spots were dried by blowing hot air and the paper strips were then chromatographed by downward displacement using the solvent mixture ethyl acetate (3)/acetic acid (3)/water (1). After eight hours the paper strips were dried and the position of the separated sugars determined by cutting a thin strip off the paper chromatogram, spraying this with aniline oxalate and heating. The chromatogram was then cut in the appropriate positions containing individual sugars (i.e., arabin-:ose and glucose) which had separated. The paper strips were eluted with water and the appropriate eluates were combined. The two solutions (of arabinose and glucose) thus obtained were then dried in a vacuum desiccator over silica gel. Very pale yellow syrups were obtained from both solutions. Examination of these by paper chromatography indicated that the two sugars were not com-

:pletely free from each other. So the sugar syrups were again chromatographed on thick filter paper as before (except that the solvent mixture, n-butanol (2)/acetic acid (1)/water (1), was used and the period of run was twenty four hours) and by doing this twice a complete separation was achieved. It was evident from the yellowish colour of the solutions that impurities were still present, but further purification proved difficult. However, on removing the water from the sugar solutions, very pale yellow syrups were obtained.

An attempt was made to crystallise the syrups from aqueous ethanol or methanol, but this was not successful. The yellow colour of the sugar solutions made it impossible to deter-:mine the optical rotation. However, it was possible to charact-:erise arabinose by making its benzoyl hydrazone derivative.

Preparation of the benzoyl hydrazone derivative of arabinose[34]

Benzoyl hydrazine (1 g., m.p. 110-112° on recrystallis-:ation from water) was dissolved in ethanol (95%, 20 ml.). The sugar syrup (30 mg.) was dissolved in water (1 ml.) and the reagent (5 ml.) added. The mixture was kept with occasional shaking at the room temperature for 24 hours and then at 0°C for 40 hours. The crystalline derivative formed had m.p. 184-187° with decomposition (Lit. m.p. 184-188°, decomp.). Mixed melting point with an authentic specimen was 182-186° (decomp.).

Attempted preparation of the p-nitroanilide derivative of glucose[35]

Conc. HCl (0.14 ml.) was dissolved in methanol (200 ml.). The sugar syrup (48 mg.) was mixed with p-nitroaniline (38 mg.) and the mixture was dissolved in the minimum quantity of the reagent by warming on the water bath. No crystalline derivative was obtained.

Caustic potash fusion of D-glucopyranosyl benzene

A mixture of tetra acetyl-D-glucopyranosyl benzene (4.3 g.) dilute sulphuric acid (5 ml.) and water (200 ml.) was refluxed until all the solid went into solution. The solution was cooled, filtered, and the filtrate evaporated to complete dryness by distillation under reduced pressure. The residue was a thick colourless syrup which solidified on adding 95% ethanol (250 ml.). The solid (D-glucopyranosyl benzene) was filtered and dried.

D-glucopyranosyl benzene (1.4 g.) and caustic potash (1 g.) were thoroughly mixed in a mortar. The mixture in a micro glass retort whose neck was bent in the form of a U-tube was strongly heated and the distillate collected in the U-tube. Examination of the distillate by gas chromatography (by Dr. W.H. Taylor) did not show the presence of toluene.

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